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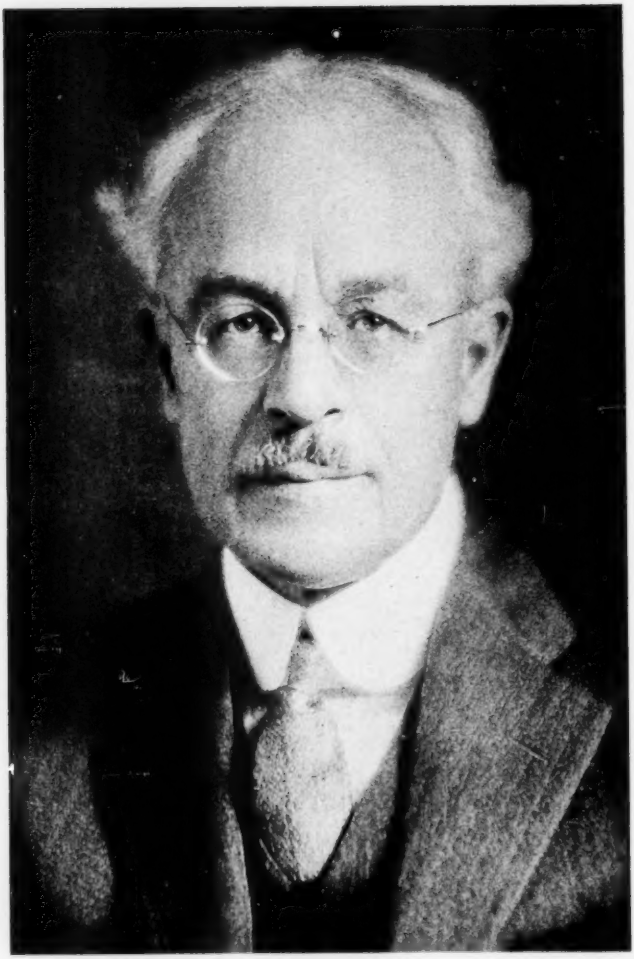

Bradley M. Davis

BRADLEY MOORE DAVIS

BRADLEY MOORE DAVIS was born in Chicago, Illinois, November 19, 1871, and died in Portland, Oregon, March 13, 1957. As a youth, he showed a strong inclination toward natural history. His activities so impressed DAVID STARR JORDAN, with whom he had become acquainted that, as a high school student, he was invited to join an ichthyological expedition into Colorado and Utah, led by JORDAN and BARTON W. EVERMAN. JORDAN was then President of Indiana University, and as a result young DAVIS enrolled at that institution, where his interests soon shifted from zoology to botany under the influence of one of his botanical instructors, DOUGLAS H. CAMPBELL. When JORDAN left Indiana to become the first president of Leland Stanford University, taking CAMPBELL with him, DAVIS and several other students accompanied them, and as a result he graduated in the first class of Stanford University.

In 1895 DAVIS received his doctorate at Harvard under FARLOW. The next eleven years were spent at the University of Chicago, where he inaugurated the work in beginning botany and plant morphology. Two summers during this period were spent at the Naples Zoological Station, and several months were devoted to study in STRASBURGER's laboratory at Bonn. In 1891 he began his association with the Marine Biological Laboratory at Woods Hole, where he worked with PROFESSOR SETCHELL. From 1897 to 1906 he was in charge of the botany course at that institution. From 1906 to 1911 he made Woods Hole his headquarters while he devoted his attention to the preparation of the well known textbook of botany by BERGEN and DAVIS, and to the investigations which resulted in a monograph of the algae of the Woods Hole region, published under the auspices of the U. S. Bureau of Fisheries. In 1911 he joined the staff of the Botany Department of the University of Pennsylvania, where he remained until 1919 when he accepted a professorship of botany at the University of Michigan. The remainder of his active career was spent at that institution.

DR. DAVIS' research activities, culminating in some 82 articles and books, were divided into two rather distinct periods. In his earlier days he was primarily interested in the algae and fungi, especially in their cytology and life histories. His contributions in this field, and those of some of his students, notably SHIGEO YAMANOUCHI, were epoch making. After leaving Chicago, his interests gradually shifted in the direction of genetics and evolution. He published some of the earliest studies on the cytology and genetics of Oenothera. As a result of these studies he became greatly interested in the question then being debated as to the origin and nature of *Oenothera lamarckiana*, upon which DE VRIES had largely based his Mutation Theory. Was it a pure species, of American origin, as maintained by DE VRIES, or was it the result of hybridization, possibly in Europe, between diverse races which had been introduced from America? DAVIS' studies inclined him toward the latter hypothesis. In order to test this concept he began to search for materials which, when crossed, would yield *lamarckiana* or something close

to it. He succeeded ultimately in producing a hybrid with a phenotype closely resembling the disputed race, which he called *neo-lamarckiana*. This hybrid differed, however, in one important respect from *lamarckiana*—it had an unbalanced lethal situation, one of its genomes being alethal. As a result, it failed to breed true but threw in every generation a class of alethal progeny. DAVIS devoted the closing years of his research career to an attempt to produce by experimental means a lethal in the alethal genome. Although he failed in this effort, subsequent work has shown that the hybrid which he produced was undoubtedly similar to *lamarckiana*, in that the parental complexes which he combined were closely related phylogenetically to those which are found in *lamarckiana*. His work, therefore, served to support the hypothesis of the hybrid origin of *lamarckiana*, which later work has also tended to confirm.

DR. DAVIS was a public-spirited member of the biological fraternity. He took an active and lively interest in all matters which affected the welfare of biology and biologists. He accepted his share of the chores, and won his share of the honors. From 1913 to 1919 he served as secretary of the American Society of Naturalists, became its vice-president in 1920 and its president in 1921. He was active in the affairs of the Botanical Society of America, and in the Genetics Society of America and the Joint Genetics Sections which preceded it. He was elected to the American Philosophical Society in 1914, was made its secretary in 1918, and served in this capacity until he left Philadelphia. During World War I he worked under HERBERT HOOVER as editor of the Statistical Division of the Food Administration. He was an original member of the Editorial Board of "Genetics" and remained a member of this group until his death, 41 years later.

An active and meticulous investigator, an enthusiastic teacher, a man of broad culture and discriminating taste, sophisticated and urbane yet approachable and unaffected, he was held in the highest esteem by all who knew him, both as a man and as a contributor of importance to the fields of cytogenetics and evolution.

RALPH E. CLELAND

SELECTION FOR DEVELOPMENTAL RATE IN DROSOPHILA PSEUDOOBSCURA[1]

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First received December 8, 1956

RECENT experimental work, showing that selective forces act upon the genotype as a coadapted system, has also revealed some unexpectedly complex situations. Thus, DOBZHANSKY and PAVLOVSKY (1953) and LEVINE (1955) found that the outcome of some replicate selection experiments has been variable. It is possible that such "indeterminacy" might be more widespread in selection experiments than is generally believed. Most laboratory studies of selection have analyzed the genetic changes in single populations. Relatively rarely have more than one or two lines from the same stock been selected for any one character. With this in mind, a replicated selection experiment was started using *D. pseudoobscura* as the experimental organism and time of development from egg to adult as a "character." The question at issue is: Does selection applied to similar populations, using similar techniques, result in identical or closely similar responses?

MATERIALS AND METHODS

Thirty lines were made from a foundation population derived from 32 strains of *D. pseudoobscura*, all but one of which were homozygous for the Arrowhead gene arrangement and each of which descended from single females collected at Piñon Flats, Mt. San Jacinto, California (16 strains), and Black Canyon of the Gunnison National Monument, Colorado (16 strains). A single Piñon strain was homozygous for the Standard gene arrangement. To provide sufficient genetic variation, females from Piñon were crossed, *en masse*, with Gunnison males; the reciprocal cross was also made. As the first F_1 flies from these crosses emerged, 100 flies, equally divided as to sex, from each of the two reciprocal crosses, were taken; the 200 adults thus secured were pooled and for each of the ten lines to be selected for increased rate of development ten males and ten females were taken at random to be the progenitors of a line. The ten selection lines, denoted from A to J, were then maintained separately.

In a like manner, 200 flies from the middle of the emergence period of the F_1 were collected to be the parents of the ten unselected control lines, which were labeled with lower case letters a to j. Similarly, 200 flies from the end of the emergence period were collected to be used as parents of the ten lines, designated Q to Z, to be selected for decreased rate of development.

[1] Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University, New York City, 1956.

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The 20 parents of each line were placed in half pint milk bottles containing lightly-yeasted Cream of Wheat-molasses food and the females were allowed to oviposit for about 24 hours at 21°C. The parents were then transferred without etherization into a second bottle, and 24 hours later to a third bottle for another 24 hour oviposition period, after which the parents were discarded. Consequently, three replicate cultures were prepared for each line. The culture bottles with the eggs deposited in them were kept at about 25°C and the flies allowed to develop.

In each of the ten fast lines, the first (or as near to the first as possible) ten males and ten females, hatching in one or more cultures that appeared to have sufficient numbers of larvae, were isolated to serve as the parents of the next generation. In the slow lines, approximately the last ten flies of each sex were selected as parents of the subsequent generation. In the unselected control lines, all of the emerging flies were collected and saved until the end of the eclosion period, when the 20 parents were taken at random for the next generation.

In each generation all of the lines within a single group (i.e. fast, slow, or control) were started at the same time to ensure reasonable uniformity of conditions. However, because of the divergence of the three groups of lines, similar generation numbers of the experimental groups do not correspond in time. At intervals, attempts were made to start two or three groups (20 to 30 lines) simultaneously so that they would be contemporary.

In generations 17, 18, and 27 of the slow, control, and fast lines respectively, determinations were made of the speed of development under less competitive conditions. Flies were placed in quart bottles and allowed to oviposit on paper spoons with standard medium. Freshly-hatched larvae were transferred in groups of ten into vials containing yeasted standard medium. The time of eclosion of the adults in these vials was recorded.

In the present experiments, developmental time means the number of days elapsed between egg deposition and the eclosion of the imago. For a group of eggs laid by several females in a culture bottle, the developmental time is the average of the individual times. As adults emerged, cultures were counted usually every second day. The results indicate that this method was satisfactory for observing the progress of the experiment.

The values in the tables are for both sexes combined.

EXPERIMENTAL RESULTS

Foundation populations

Because of unusually high larval densities in the F_1 of the interstrain crosses, a better determination of the time of development at the outset of the experiment may be had from the first selected generations of the lines. See Table 1 and Figure 1. Despite the considerable variation, analyses of variance show no statistically significant differences in time of development among the groups and among the lines within each group: unselected control lines, F (9, 11) = 1.01;

fast-selected lines, F (9, 16) = 1.28; slow-selected lines, F (9, 15) = 0.18; all lines, F (2, 69) = 0.37. For each F value, P is greater than .05.

Unselected control lines

The behavior of this unselected group is shown in Table 1A and in Figure 1. Except for line b, there are no clear-cut trends. This is confirmed when regression functions are computed both for the lines separately and for the within-generation means of all ten lines together, assuming that the data can be fitted to a straight line. Estimates of the linear regression parameters (up to the 17th generation) are shown in Table 2A together with tests of the deviations of the

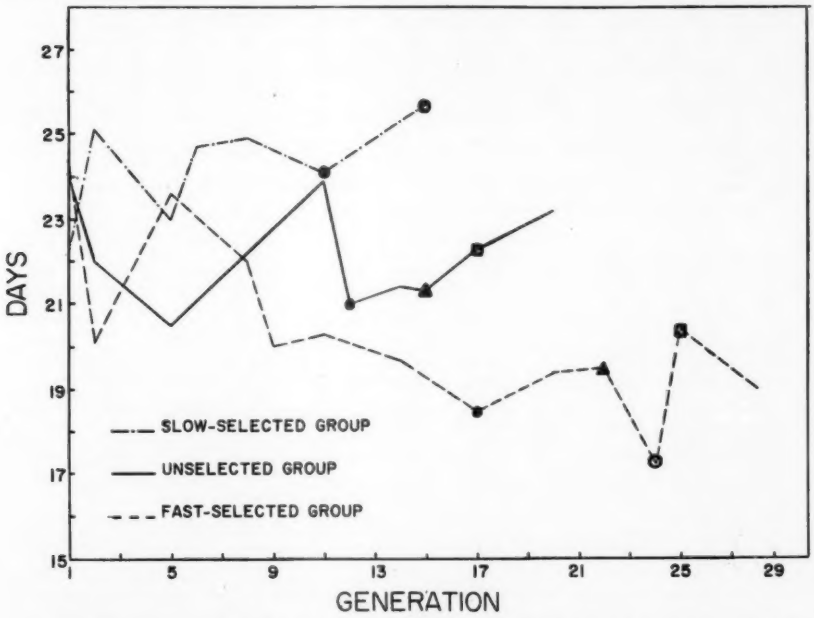

FIGURE 1.—Responses to selection for developmental rate in *Drosophila pseudoobscura*. The numbers of days of development from egg to imago are based on the means of (a) ten replicate lines selected for faster development, (b) ten replicate lines selected for slower development, and (c) ten unselected replicate lines serving as controls. The contemporaneity of the three groups is not to be judged from the generation numbers since they were not raised simultaneously except as follows: slow, control, and fast— generations 11, 12, and 17, respectively, indicated on the graph by solid circles; control and fast— 15, 22, indicated by solid triangles; slow and fast— 15, 24, open circles; control and fast— 17, 25, open squares.

regression coefficients from zero. None of the coefficients differs significantly from zero, not even that for line b which appeared to be accelerating its development. Six of the coefficients are negative and four positive, about what might be

TABLE 1

Mean developmental time in days from egg to adult of 30 experimental strains of Drosophila pseudoobscura

	(A) Ten replicate unselected control lines									
	Lines									
Generation	a	b	c	d	e	f	g	h	i	j
1	22.74	24.62	25.27	21.00	26.81	21.80	24.88	23.11	24.70	26.31
2	20.51	23.23	19.49	23.57	20.32	20.96	23.02	21.07	23.27	23.74
5	22.18	20.80	22.20	20.63	19.02	21.28	20.43	19.80	18.93	20.36
8	....	19.80	24.85	20.36	24.85	20.26	20.76	23.97	19.88	24.00
11	23.79	20.13	25.78	25.52	23.11	24.57	22.50	24.77	28.03	26.35
12	21.51	18.66	23.25	19.57	20.85	20.98	19.81	21.64	21.29	21.61
14	23.18	16.99	23.42	19.86	20.35	22.82	22.78	21.66	19.37	22.22
15	25.21	17.62	20.74	20.30	20.46	22.94	20.93	22.87	22.97	19.77
17	20.39	22.44	22.69	22.34	22.86	21.34	20.93	25.80	21.93	24.73
20	24.85	21.05	22.73	22.96	24.84	22.58	21.89	23.59	26.35	23.04

	(B) Ten replicate lines selected for fast development									
	Lines									
Generation	A	B	C	D	E	F	G	H	I	J
1	21.00	23.26	24.89	25.09	23.46	23.61	25.39	24.65	27.71	25.82
2	21.22	19.13	19.46	19.87	21.61	18.29	20.86	21.14	20.27	20.25
5	25.08	22.84	22.41	26.06	23.82	22.77	24.21	21.38	25.32	22.92
8	23.01	23.55	19.38	....	23.37	....	22.93	21.08	....	20.00
9	23.36	22.04	....	20.20	17.47	17.85	20.47	....	....	22.33
11	24.64	23.08	21.55	18.37	20.58	16.58	19.23	16.94	19.08	19.82
14	19.57	16.73	20.55	15.49	25.76	....	18.17	16.99	16.80	21.12
17	21.50	19.58	18.04	16.50	19.42	17.95	18.10	14.83	19.05	17.08
20	24.04	20.24	....	....	18.64	....	19.60	....	17.44	17.90
22	26.31	19.47	18.33	19.05	18.25	....	19.46	18.49	18.72	18.80
23	21.94	20.20	14.65	....	17.57	....	18.42	14.03	....	....
24	20.13	....	15.60	17.05	....	....	19.63	16.21	17.36	18.53
25	23.77	22.90	20.73	17.16	....	....	19.06	18.41	18.17	23.31
28	23.76	15.85	20.03	....	17.25	....	21.20	....	....	18.56

	(C) Ten replicate lines selected for slow development									
	Lines									
Generation	Q	R	S	T	U	V	W	X	Y	Z
1	20.03	22.45	21.82	22.77	22.39	22.35	23.33	23.78	21.15	21.22
2	23.15	24.17	26.02	27.66	18.87	23.42	26.53	27.56	24.79	26.66
5	....	23.07	23.80	22.43	22.49	23.09	20.07	24.91	24.32	22.80
6	23.67	25.34	27.13	22.94	22.53	28.44	....	24.03	25.39	22.24
8	20.38	25.58	26.00	20.36	27.14	24.19	24.99	26.72	24.28	25.79
11	23.78	28.52	23.27	25.91	24.72	23.82	22.48	24.46	21.08	25.59
14	....	23.47	....	25.82	....	....	....	28.29	23.38	....
15	24.49	24.62	....	23.78	23.36	....	27.28	25.58	....	33.04

TABLE 2

Estimates of regression parameters and test of significance of regression coefficients

Line	(A) Unselected control lines*			
	b	*t*	d.f.	P
a	0.061	0.568	6	.5–.6
b	—0.293	—2.31	7	.05–.1
c	0.0008	0.0006	7	> .9
d	—0.046	—1.12	7	≃ .3
e	—0.112	—0.697	7	.5–.6
f	0.077	0.937	7	.3–.4
g	—0.144	—1.59	7	.1–.2
h	0.150	1.35	7	.2–.3
i	—0.006	—0.003	7	> .9
j	—0.112	—0.742	7	.4–.5
Group	—0.006	—0.071	7	> .9

Line	(B) Fast-selected lines†			
	b	*t*	d.f.	P
A	0.023	0.321	11	.7–.8
B	—0.074	—0.945	10	.3–.4
C	—0.226	—2.89	9	.01–.02
D	—0.280	—2.79	8	.02–.05
E	—0.219	—2.23	9	.05–.1
F	—0.303	—1.62	4	.1–.2
G	—0.201	—3.61	11	< .01
H	—0.275	—3.64	9	< .01
I	—0.305	—3.30	8	.01–.02
J	—0.151	—1.83	10	.05–.1
Group	—0.175	—3.68	11	< .01

Line	(C) Slow-selected lines			
	b	*t*	d.f.	P
Q	0.205	1.42	4	.2–.3
R	0.136	0.985	6	.3–.4
S	0.078	0.913	4	.4–.5
T	0.044	0.024	6	> .9
U	0.271	1.40	5	.2–.3
V	0.167	0.602	4	.5–.6
W	0.140	0.610	4	.5–.6
X	0.099	0.789	6	.4–.5
Y	—0.043	—0.262	5	≃ .8
Z	0.608	2.61	5	≃ .05
Group	0.145	1.61	5	.1–.2

* Computed up to and including generation 17.
† Computations do not include generation 28.

expected if no directional forces were acting similarly upon all of the lines. Up to generation 17, the regression line fitted to the means for the group of ten lines has a slight downward slope, but the regression coefficient, —0.006, is not significantly different from zero ($P > .9$).

Do the lines show significantly different trends? While none of the regression coefficients differs from zero, it is possible that the extreme lines, at least, have coefficients that differ significantly from each other. However, this cannot be tested by a customary t or F test. These tests are valid for testing the differences between two or more lines specified in advance, but if they are used to test the lines observed to differ most, "significant" differences would be found by chance more than five percent of the time. Accordingly, Dr. Howard Levene has suggested a test, based on Scheffé (1953), which enables the testing of any differences suggested by the data. If no true differences exist, then only five percent of the time will one or more of the selected differences be significant at the five percent level. Combinations of regression coefficients are taken two at a time, the statistic g is computed, and if the difference between a pair of coefficients exceeds g, then the coefficients are significantly different from each other at a chosen level of significance, where

(1) $$g = (s^2 S^2 \Sigma a_i^2)^{1/2},$$

(2) $s^2 = s^2_{y \cdot x}$, a pooled estimate of σ^2, with $n = \Sigma(N_i - 1)$ degrees of freedom,

(3) $$S^2 = (k - 1) \cdot F\alpha\,(k - 1, n);\ k = \text{number of lines},\ \alpha = .05,$$

and

(4) $$a_i^2 = 1/\Sigma(x_{ij} - \overline{X}_i)^2.$$

For all of the 45 possible comparisons, g has a value of 0.40. The only difference that exceeds this value is the one between lines b and h, which is 0.44.

In generation 18, larvae were collected from each line and placed in three vials, with ten larvae per vial, and the developmental time in hours recorded (Table 3A). While these rates are not exactly the same as the rates from the crowded culture bottles, they should reveal differences among the lines if such exist. An analysis of variance shows that there are no significant differences among the lines; $F\ (9, 18) = 1.28$, $P > .05$. Line b, which previously appeared anomalous, now appears to be very near the mean of the control group; the grand mean is 360 hours and the mean of b is 357 hours.

It may be concluded that, with the doubtful exception of line b, there have been no statistically significant changes in the control lines and that at generation 18 none of the lines differed significantly in developmental rate from the others.

Fast-selected lines

The outcome of selection for the reduction of developmental time is shown in Tables 1B and 2B, and in Figure 1. After 28 generations of selection, the average decrease in development time is between two and four days by comparison either with the first generation of selection or with the unselected controls. The fast-selected lines as a group fluctuate around a value of about 19 days. This situation

TABLE 3

Developmental time in hours of experimental strains of Drosophila pseudoobscura, *estimated from data of vials*

(A) Unselected control lines, generation 18		(B) Fast-selected lines, generation 27	
Line	Mean	Line	Mean
a	374.0	A	343.1
b	357.0	B	349.9
c	352.1	D	344.4
d	363.5	E	335.8
e	353.7	G	325.4
f	363.8	H	317.7
g	340.3	I	328.1
h	378.2	J	329.3
i	345.4		
j	361.7		
Group	360.3	Group	334.2

(C) Slow-selected lines, generation 17		(D) Slow-selected lines, generation 17*	
Line	Mean	Line	Mean
Q	364.0	Q	366.4
R	398.8	R	347.0
U	373.9	T	300.8
V	351.1	U	367.0
W	355.4	V	320.5
X	393.4	W	314.3
Y	347.1	X	332.8
		Y	306.3
Group	370.0	Group	331.9

* Not determined synchronously with estimates A, B, and C.

may have been reached by generation 14, or at most by generation 17. The decline of development time is apparent after generation 8, so that most of the observed change took place within relatively few generations. The group regression coefficient, —0.175, is significantly different from zero, $P < .01$.

At four different points the fast-selected lines were reared contemporaneously with one or both of the two other experimental groups, thus minimizing macroenvironmental differences among them. These points are indicated in Figure 1. At generation 17, the fast lines developed on the average 2.5 days faster than generation 12 of the controls, and 5.5 faster than generation 11 of the slow-selected lines. At generation 22, the fast lines were 1.5 days faster than the 15th control generation, and at generation 25 they were two days faster than the 17th control generation. Finally, at the 24th generation, the fast lines developed approximately eight days faster than the slow lines, then in generation 15. These differences are all significant at the five percent level or below. For generation 17, fast group, and generation 12, control group, $F\ (1, 70) = 19.5$; for generation

22, fast group, and generation 15, controls, F (1, 27) = 4.25; for generation 25, fast group, and generation 17, controls, F (1, 64) = 7.20; for generation 17, fast group, and generation 11, slow group, F (1, 66) = 62.1; and for generation 24, fast group, and generation 15, slow group, F (1, 22) = 66.9. These differences, not present at the beginning of selection, and which appear under reasonably uniform conditions, suggest that there is a genetic differentiation in developmental rate among the experimental groups.

Apparently not all of the lines have contributed equally to the observed change in mean rate of development. Indeed, some lines appear not to have responded to selection at all, while others responded quite strongly. Comparison of the nine lines which survived to the 25th generation shows that five of them have regression coefficients significantly different from zero at the five percent level or below (Table 2B). Of the four remaining lines, two appear to have responded somewhat to selection (E and J), whereas lines A and B showed no response whatsoever. Line F was accidently lost after generation 17; until then, it appeared to increase its development rate, but the regression coefficient is not significant.

The regression coefficients may be examined further for differences among them by the method discussed in the preceding section. For the 36 paired comparisons, the value of g varies between 0.22 and 0.25. (The extinct line, F, was not included in this analysis.) The only significant differences are those between the unresponsive line A and lines C, D, E, G, H, and I, a not unexpected result. The difference between lines B and I falls short of significance by 0.001. The only other differences that are at all close to their calculated g value are those between B and the two next most extreme lines, D and H. These results confirm that A and B have not responded to selection and that the lines with the largest regression coefficients have. Otherwise, the magnitude of the responses in seven of these lines are essentially alike.

In generation 27, the speed of development in vials was studied. The data for eight of the nine lines of the group (only eight have sufficient larvae for the test) indicates that the average developmental time of the fast-selected lines is 334 hours (Table 3B). The difference between this value and 360 hours found for the controls at the same time is highly significant; $t_{16} = 4.80$, $P < .001$. Analysis of variance suggests that differences may exist among the lines; F (7, 16) = 2.70, $P = .05$. Since the means of the individual lines may not be equal, a method for judging all differences, while still maintaining the proper level of significance, may be used to find which means, if any, really differ. This is based on a procedure of TUKEY (see SCHEFFÉ 1953), which is more sensitive than SCHEFFÉ's method used above, but is unsuited to testing the regression coefficients. The standard deviation is multiplied by the upper five percent point of the Studentized range for eight (number of samples) and 16 (error mean square) degrees of freedom, to give a value with which to compare the difference between any two means. The standard deviation is the square root of the error mean square divided by three, the number of observations for each mean. Any difference which exceeds the computed value is significant. This value for the fast-

selected group is 32.7. The largest difference observed, 32.2, is that between lines B and H. Consequently, it appears that the means are all essentially alike. Since F barely exceeded the critical value (2.66) at the five percent level, there is reason to doubt that there are real differences among the means, although from the regression analysis such differences are expected.

The developmental times measured in vials are somewhat consistent with the estimates of change reflected in the regression coefficients, but a rank-correlation test does not show significant agreement between the two rankings; $r_s = .38$, $N = 8$, $P > .05$. However, this does not show an absence of a real relationship between them.

A response to selection is seen in the fast-selected lines, where eight of the ten show some advance in the expected direction. While there is evidence of a productivity decline, it cannot be wholly responsible for the observed increases of developmental rate. In contrast to six out of ten negative, but nonsignificant coefficients in the controls, which also declined somewhat in productivity, here nine of the ten coefficients are negative and five of these differ significantly from zero. Furthermore, although the weakly and nonresponding lines are included in the computation of a single regression function for the group of lines, the regression coefficient differs significantly from zero. Finally, under relatively uniform conditions and with a small, standardized number of larvae, significant differences persist between the control and fast-selected groups: now about two days in culture bottles, one day in vials.

Although an analysis of variance of developmental time in experimental vials suggests the existence of differences among lines, an additional statistical test does not show these differences to be quite significant.

Slow-selected lines

The experimental results are given in Tables 1C and 2C. There is an apparent retardation in most of the lines, except line Y, but none of the regression coefficients differ significantly from zero. Even the high coefficient of line Z is of doubtful significance; the apparently good response is probably mainly caused by the unusually slow development of the last generation, in all likelihood an environmental effect. The response of the group as a whole is small; the regression coefficient, 0.090, is nonsignificant.

Nevertheless, all but one of the coefficients are positive, suggesting a slight upward trend. The slow lines were twice reared contemporaneously with one or both of the other groups. In generation 11 they developed on the average in 24 days, while the 12th generation of controls took 21 days, a three day difference under similar conditions; $F\ (1, 66) = 20.99$, $P < .01$. At the same time, the 17th generation of the fast group developed in 18.5 days. In generation 15, the figure for the slow group was 25.7 days; the fast group, then in generation 24, developed eight days faster. Thus, there is some justification in suggesting that a small increase in development time may have taken place in the slow lines.

The different lines may be compared with respect to their rates of advance

under selection. The values of g for the 45 comparisons vary between 0.50 and 0.82. As might be expected, only the most extreme lines, T and Y, differ significantly from Z.

The data in Table 3C, on developmental time in vials in generation 17, are based on flies that developed synchronously with flies from the two other groups and can be compared with Tables 3A and 3B. The difference from the fast-selected lines is clear; the only overlap is that of lines B and Y, both of which did not respond to selection. The overlap with the controls is considerable, however, and the small mean difference of ten hours between the two groups is not significant; $t_{15} = 1.18$, $P > .05$. Unfortunately, lines S, T, and Z did not produce enough larvae for the test. Some of the slowest lines in the test vials are also among the lines with the largest positive regression coefficients, but a rank-correlation test showed that the agreement was not significant; $r_s = .11$, $N = 7$, $P > .05$.

A second series of vials was set up a few days after the first series. Both sets of larvae thus were developing concurrently for the greater part of their larval periods. The importance of whatever environmental differences existed is apparent when Table 3C is compared with Table 3D. The observations are widely disparate. The new data suggest that the slow-selected flies develop at least as rapidly as the fast-selected flies, although this is not true when the two groups are tested simultaneously. With this in mind, the fluctuations in development rate appearing in these experiments are best interpreted as being mainly caused by both macro- and microenvironmental variation among cultures, among lines, and among generations. It is possible too that real responses in the slow-selected group are hidden by the large fluctuations.

Although again no data on line Z were procured, this time a rank-correlation test indicates significant agreement between the ranking based on regression coefficients and that based on the test vials; $r_s = .786$, $N = 8$, $.05 > P > .01$.

Analyses of variance of the data in Tables 3C and 3D indicate significant differences among lines; $F\ (6, 12) = 8.21$, $F\ (7, 16) = 6.22$, $P < .01$. All pairs which differ by more than 34.2 hours in Table 3C and 51.0 hours in Table 3D are significantly different, the values of 34 and 51 hours being calculated by the method discussed in the preceding section. In Table 3C, the pairs which so differ are RV, RW, RQ, XV, and XW. (Lines U and Y were not used in these comparisons because only two replicates were secured for them.) In Table 3D, the differing pairs are UT, UY, UW, QT, QY, and QW. These results suggest that there is a group of slow lines (U, X, and R) which is probably different from a group of faster lines (T, V, W, and Y). This is further reason to suspect that there may be some real responses to selection, despite the absence of significant regression coefficients and the failure to find significant differences among them.

The slow-selected lines give a general impression that they have suffered some physiological upsets not so apparent in the other groups. The emergence period often has been prolonged and erratic, with many larvae dying despite no evidence of overcrowding. This was especially true in line Z. The causes of these

phenomena are unknown, but it seems possible that selection in the slow lines could be partly for deleterious genes that almost stop development altogether, prolonging emergence. Metabolic upset is more likely to be accompanied by delayed emergence than by rapid emergence. This, plus differences in the amount of selection applied to the slow and fast groups, might account in part for the asymmetrical response to selection.

At first sight, the slow-selected lines appear not to have responded to selection since, with one doubtful exception, the regression coefficients are all nonsignificant. However, nine of the ten lines have positive coefficients, in contrast to four of ten in the control group and one of ten in the fast group. At the very least, selection prevented any increase in development rates, although the productivity decline that occurred should have resulted in some spurious increases. Furthermore, the standard errors of the regression coefficients tend to be larger in these lines than in the other experimental lines. Consequently, it may be that some real responses to selection are present, but that they are not very marked and are masked by both the large variance and to an unknown extent by the environmental countereffect of smaller numbers of larvae in cultures. Evidence from the experimental vials is consistent with the hypothesis that a small increase in developmental time occurred, although the half-day mean difference between the slow group and the controls is not significant. The analyses of two independent sets of data from the vials indicate that there are differences among the lines. Since none of them accelerated, this divergence was probably achieved by retardation of some of the lines.

While there are no significant differences among the regression coefficients, the differences among the lines at the end point, as tested in the vials, is suggestive of nonidentical results, or variability in the outcome of selection.

DISCUSSION

Attention has recently been directed to variability in selection response by DOBZHANSKY and PAVLOVSKY (1953) and LEVINE (1955) who compared the outcomes of replicate experiments on natural selection of chromosomal polymorphs in populations of *D. pseudoobscura*. In populations of uniform geographic origin the results were "determinate," since similar equilibria were reached after approximately the same number of generations of selection. In geographically mixed populations, as in the present experiment, the results were "indeterminate," since in some of the populations one of the competing polymorphs was eliminated, while in other populations heterotic equilibria were established at different levels and at different times. It is not possible to predict in advance which result will be observed in a given population of geographically mixed origin.

A relative "indeterminacy" in the outcome of selection programs may occur even in experiments using lines derived from flies apparently of uniform origin. ROKITZKY (1936) studied selection of sternopleural bristle number in 40 X-rayed and unirradiated *D. melanogaster* lines. Comparison of the nine "success-

ful" lines with each other reveals that the mean bristle numbers resulting from selection and the times when the response to selection became apparent varied from line to line. SISMANIDIS (1942) selected in ten lines of *D. melanogaster* for greater numbers of scutellar macrochaetae. Although derived from a single foundation stock, some selection lines showed early and marked rises in chaetae number, while others reacted more slowly. OHSAWA and TSUKUDA (1955) found differences in the response of different lines for selection for larval resistance to copper sulfate in the nutrient medium. CLAYTON, MORRIS and ROBERTSON (1954) specifically investigated the variability of selection response for numbers of chaetae on the abdominal sternites, using several lines derived from the same initial population of *D. melanogaster*. Considerable variation was found among the lines.

The experiment reported in the present article was made to investigate the variability of selection responses using a species other than *D. melanogaster* and a character other than chromosomal polymorphism, polygenic in nature, but presumably of clear ecologic importance. Some lines responded to selection for increased developmental rates, whereas others failed to change or changed only slightly. Responses to selection for slower development were less clear, but there was some evidence of differences among the lines in the last generation tested. Five among the lines selected for rapid development had regression coefficients significantly different from zero, although a statistical test showed no significant heterogeneity among the coefficients, except for one extreme line. No significant changes and differences were found among ten unselected control lines. Therefore, in this experiment too, the results of parallel selections are not always identical.

Similar end results do not necessarily mean that the responses to selection are identical. It is well known that similar phenotypes may be arrived at by diverse genetic means. This is shown by experiments such as that of KING (1955), who selected two lines, derived from the same wild population of *D. melanogaster*, for resistance to DDT. Both lines developed resistance apparently at the same rates and to the same degree, so that they were phenotypically indistinguishable. Nevertheless, crosses between the lines lead to the conclusion that different sets of genes have been selected in the two populations, yielding, however, similar phenotypes. In the present experiment, it does not follow that similarly responding lines have achieved their new rates by utilizing the same gene combinations. The data of WALLACE (1955) show that similar development rates may be produced by different kinds of heterozygotes. Thus, among our lines, increases or decreases of development rates may have been achieved by consolidating different combinations of genes.

Mendelian segregation and recombination produce innumerable gene combinations; natural selection, acting upon the genotype as a whole, favors genes which combine well with others to form adaptively valuable combinations. The concordance and interaction of the numerous factors involved in gradual reconstructions of a genotype are unlikely to be exactly duplicated. The establishment

of new integrated genetic systems and new norms of reaction is not expected generally to be repeatable or reversible. The evidence discussed in this article provides a bridge on the experimental level between genetic hypothesis and the observed record of organic evolution.

SUMMARY

Thirty strains of *D. pseudoobscura* were derived from a population resulting from a cross of flies of California and Colorado origins. Ten lines were selected for fast rates of development, ten selected for slow rates, and ten control lines were unselected. No appreciable changes in rates of development occurred in the unselected lines. Five of the fast lines gave a significant response to selection, two lines gave a weak response, two lines failed to change, and one line became extinct after a slight acceleration. The slow lines failed to show significant changes or differences from the controls; nevertheless, it seems likely that some increases in development time may have occurred. Differences in developmental rates among the slow lines were clearest in the last generation tested. This and some other experiments reported indicate that in complex genetic situations, where many segregating genes are involved, the results of selection may be variable even when replicate lines are kept under reasonably uniform conditions.

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LITERATURE CITED

CLAYTON, G., J. A. MORRIS, and A. ROBERTSON, 1954 Selection for abdominal chaetae in a large population of *Drosophila melanogaster*. IUBS Symposium on genetics of population structure. IUBS Ser. B. **15**: 7–15.

DOBZHANSKY, TH., and O. PAVLOVSKY, 1953 Indeterminate outcome of certain experiments on Drosophila populations. Evolution **7**: 198–210.

KING, J. C., 1955 Integration of the gene pool as demonstrated by resistance to DDT. Am. Naturalist **89**: 39–46.

LEVINE, L., 1955 Genotypic background and heterosis in *Drosophila pseudoobscura*. Genetics **40**: 832–849.

OHSAWA, W., and H. TSUKUDA, 1955 Effect of selection in $CuSO_4$-containing media on the larval copper resistance in Drosophila. J. Inst. Polytechnics. Osaka City Univ. D **6**: 97–105.

ROKITZKY, P., 1936 Experimental analysis of the problems of selection by X-ray irradiation. I. Comparison of the rate of selection in the lines treated by X-rays with those not treated by X-rays. Uspehi Zootehniceskih Nauk. **2**: 161–202.

SCHEFFÉ, H., 1953 A method for judging all contrasts in the analysis of variance. Biometrika **40**: 87–104.

SISMANIDIS, A., 1942 Selection for an almost invariable character in Drosophila. J. Genet. **44**: 204–215.

WALLACE, B., 1955 Interpopulation hybrids in *Drosophila melanogaster*. Evolution **9**: 302–316.

IMPATIENS BALSAMINA L.—THE INHERITANCE OF FLORAL COLORS

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THE floral colors of the garden balsam, *Impatiens balsamina* L., range from magenta to cream and white; and include red, purple, and various shades and tints of lavender and of pink. RASMUSON (1920), HAGIWARA (1926), KANNA (1926, 1934), BEDELL (1927), DAVIS (1927, 1932), and DAVIS, TAYLOR and ASH (1939) have reported on the inheritance of some or all of these colors. The present authors find the pale colors to be the key to all of the others. Previous investigators have not recognized the significance of these pale colors. In this paper, names of colors with initial capitals refer to the charts of RIDGWAY (1912).

PRESENTATION OF DATA

Data for cases involving single color genes are presented in Table 1. Cases involving two gene differences are presented in Table 2, and three gene differences in Table 3. Some of the "crosses" listed refer to single crosses, whereas others are combinations of two or more similar crosses. This has been done only when the populations showed closely similar ratios. When like crosses gave widely different ratios, each has been assigned a separate number and listed separately in the tables.

White and Noncream vs. *Cream;* W, w

Some of the plants bear pure "white" flowers. Others have flowers of pale creamy yellow which we call "cream." This color cannot be described by reference to RIDGWAY's charts. A cross of a true-breeding white with cream gives, in the F_2, a ratio of three whites to one cream (Table 1, Cross 1). The single pair of genes concerned is *W*, *w;* the *W* producing white and being dominant over *w* which, in the homozygous state, produces cream.

Although cream colored flowers are produced only in the absence of certain color factors dealt with below, the phenotypic expression of *W*, in contrast with *ww*, is fairly well distinguishable in their presence. In the genotype *ww*, the pollen and spurs have a creamy tinge which is particularly noticeable in the absence of any other genes for color. In such cases the cream color extends over the petals. When other color producing genes are associated with *ww*, the pollen has the creamy tinge, and the color of the spurs is modified from that which is characteristic of plants bearing the same color genes in combination with *W*. There is a complete noncream series of which the member containing no effective color

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genes, except *W*, is white, and a corresponding cream series of which the lowest member is cream. Crosses 15a and 22a (Table 3), involve noncream and cream and show, in the F_2, samples of color types in the two series. It can be seen that in each color class the noncream: cream ratio is essentially 3:1. The total is 615 noncreams: 180 creams in Cross 15a, and 1377:514 in Cross 22a.

Hereafter in this paper "white" will be used indifferently for white or cream unless the *W* gene is specifically involved.

Nearly-white Lavender vs. *White;* L, l

A type which is nearly white but shows a tinge of lavender is designated "nearly-white lavender." It falls, in RIDGWAY's plates, between White and Pale

TABLE 1

Crosses involving a single color gene

Number of cross	Description of parents	Description of F_1	F_1 genotype	F_2 Phenotypes 3:1 ratio				
				Colors	Obs.	Calc.	Tot.	χ^2
1	White	White	*Ww*	White	1617	1656	....	....
	Cream			Cream	591	552	2208	3.68
2		Nearly-wh. lavender	*Ll*	N.-wh. lav.	261	254	....	....
				White	78	85	339	0.77
3	Het. Hermosa	Hermosa	*Hh*	Hermosa	97	94	....	....
	White			White	29	32	126	0.38
4	N.-wh. pink	Pale nearly-white pink	$P^{g}p$	N.-wh. pink	805	819	....	....
	White			White	287	273	1092	0.96
5	Rose	Rose	$HhP^{g'}P^{g}$	Rose	2366	2410	....	....
	N.-wh. pink			N.-wh. pink	847	803	3213	3.21
6	Lavender	Lavender	$LlP^{g}P^{g}$	Lavender	809	819	....	....
	N.-wh. pink			N.-wh. pink	283	273	1092	0.49
7	Pink	Pink	$P^{r}p$	Pink	1476	1445	....	....
	White			White	450	481	1926	2.67
8	Pink	Pink	$P^{r}P^{g}$	Pink	2136	2090	....	....
	N.-wh. pink			N.-wh. pink	651	697	2787	4.05
9	Purple	Purple	$LlP^{r}P^{r}$	Purple	1147	1141	....	....
	Pink			Pink	374	380	1521	0.12
10	Purple	Purple	$LLP^{r}P^{g'}$	Purple	233	218	....	....
	Lavender			Lavender	58	73	291	4.11
11	Red	Red	$HHP^{r}p$	Red	748	729	....	....
	Hermosa			Hermosa	226	245	974	1.97
12	Red	Red	$HHP^{r}P^{g'}$	Red	2775	2756	....	....
	Rose			Rose	900	919	3675	0.52
13	Magenta	Magenta	$LLHhP^{r}P^{r}$	Magenta	404	428	....	....
	Purple			Purple	167	143	571	5.36
14	Magenta	Magenta	$LlHHP^{r}P^{r}$	Magenta	1248	1239	....	....
	Red			Red	405	414	1653	0.27

TABLE 2

Crosses involving two color genes

Number of cross	Description of parents	Description of F_1	F_1 genotype	F_2 Phenotypes 9:3:3:1 ratio				
				Colors	Obs.	Calc.	Tot.	χ^2
15	Rose	Pale rose	*HhP$^{g'}$p*	Rose	1635	1622	....	....
				N.-wh. pink	551	541	....	....
	White			Hermosa	532	541	....	....
				White	166	180	2884	....
								1.53
16	Nearly-wh. pink	Pale lavender	*LlP^{g}p*	Lavender	2235	2224	....	....
	Nearly-wh. lavender			N.-wh. pink	774	741	....	....
				N.-wh. lav.	725	742	....	....
				White	220	247	3954	....
								4.86
17	Lavender	Pale lavender	*LlP^{g}p*	Lavender	3758	3757	....	....
				N.-wh. pink	1248	1253	....	....
	White			N.-wh. lav.	1161	1252	....	....
				White	512	417	6679	....
								28.23
18	Nearly-wh. pink (deeper)	Lavender	*LlP$^{g'}$p*	Lavender	977	976	....	....
				N.-wh.-pink	321	326	....	....
	Nearly-wh. lavender			N.-wh. lav.	321	326	....	....
				White	117	108	1736	....
								0.91
19	Nearly-wh. pink (v.deep)	Deep lavender	*LlP$^{g''}$p*	Lavender	940	885	....	....
				N.-wh. pink	258	295	....	....
	Nearly-white lavender			N.-wh. lav.	258	295	....	....
				White	117	98	1572	....
								16.39
20	Lavender	Rose-lavender	*LlHhP$^{g'}$P^{g}*	Rose-lav. / Lavender	836	880	....	....
	Rose			Rose	266	220	....	....
				N.-wh. pink	71	73	1173	....
								11.90
21	Pink	Purple	*LlP^{r}p*	Purple	88	87	....	....
				Pink	27	29	....	....
	Nearly-white lavender			N.-wh. lav.	32	29	....	....
				White	8	10	155	....
								.86
21a	Pink	Purple	*LlP$^{r'}$p*	Purple	400	431	....	....
				Pink	160	144	....	....
	Nearly-white lavender			N.-wh. lav.	145	144	....	....
				White	62	48	767	....
								8.10
21b	Pink	Purple	*LlP$^{r''}$p*	Purple	2959	2879	....	....
				Pink	881	959	....	....
	Nearly-white lavender			N.-wh. lav.	1004	960	....	....
				White	274	320	5118	....
								17.20

TABLE 2—(Continued)

22	Pink	Purple	*LlPrPg*	Purple	1048	1064	....	....
				Pink	377	354	....	....
	Lavender			Lavender	355	355	....	....
				N. wh. pink	111	118	1891	....
								2.14
23	Purple	Purple	*LlPrp*	Purple	1790	1742	....	....
				Pink	547	581	....	....
	White			N.-wh. lav.	548	581	....	....
				White	213	194	3098	....
								7.03
24	Purple	Purple	*LlPrp*	Purple	1065	1029	....	....
				Pink	326	343	....	....
	White			N.-wh. lav. / White	438	457	1829	....
								2.89
25	Purple	Purple	*LlPrPg*	Purple	2340	2317	....	....
				Pink	776	773	....	....
	Nearly-white pink			Lavender	736	772	....	....
				N.-wh. pink	268	258	4120	....
								2.31
26	Red	Red	*HhPrp*	Red	1419	1441	....	....
				Pink	523	480	....	....
	White			Hermosa	449	480	....	....
				White	170	160	2561	....
								8.91
27	Pink	Red	*HhPrPg'*	Red	227	206	....	....
				Pink	44	69	....	....
	Rose			Rose	79	68	....	....
				N.-wh. pink	16	23	366	....
								15.09
27a	Pink	Red	*HhPr'Pg'*	Red	2484	2354	....	....
				Pink	734	785	....	....
	Rose			Rose	712	785	....	....
				N.-wh.-pink	256	262	4186	....
								17.45
27b	Pink	Red	*HhPr''Pg'*	Red	2246	2166	....	....
				Pink	745	722	....	....
	Rose			Rose	594	722	....	....
				N.-wh. pink	266	241	3851	....
								28.99
28	Magenta	Magenta	*LlHhPrPr*	Magenta	85	95	....	....
				Purple	26	32	....	....
	Pink			Red	39	32	....	....
				Pink	20	11	170	....
								11.07

TABLE 2—(Continued)

29	Magenta	Magenta	*LlHHP^{r}P$^{s'}$*	Magenta	3089	2964	....	....
				Red	945	988	....	....
	Rose			Rose-lav.	936	988	....	....
				Rose	299	329	5269	....
								13.62
30	Purple	Magenta	*LlHhP^{r}P^r*	Magenta	142	152	....	....
				Purple	56	51	....	....
	Red			Red	56	51	....	....
				Pink	17	17	271	....
								1.64

TABLE 3

Crosses involving three color genes

Number of cross	Description of parents	Description of F_1	F_1 genotype	F_2 Phenotypes 27:9:9:9:3:3:3:1 ratio				
				Colors	Obs.	Calc.	Tot.	χ^2
15a	Rose,	Pale rose,	*HhP$^{s'}$pWw*	Rose, n-cr.	348	336	....	....
	noncream	noncream		Rose, cream	100	112	....	....
(Cf. 15,				Nearly-white				
Table 2)				pink, non-cr.	106	112	....	....
				Nearly-white				
				pink, cream	35	37	....	....
	Cream			Hermosa, n-cr.	124	112	....	....
				Hermosa, cr.	33	37	....	....
				White	37	37	....	....
				Cream	12	12	795	....
								3.86
22a	Lavender,	Purple,	*LlP^{r}P^{s}Ww*	Purple, n-cr.	754	797	....	....
	cream	noncream		Purple, cream	294	266	....	....
(Cf. 22,				Pink, n-cr.	270	266	....	....
Table 2)				Pink, cream	107	89	....	....
	Pink,			Lav. n-cr.	270	266	....	....
	noncream			Lav. cream	85	89	....	....
				Nearly-white				
				pink, n-cr.	83	89	....	....
				Nearly-white				
				pink, cream	28	29	1891	....
								9.64
31	Red	Magenta	*LlHhP^{r}p*	Magenta	503	466	....	....
				Purple	113	155	....	....
				Red	162	155	....	....
				Pink	41	52	....	....
	Nearly-white			Hermosa-lav.				
	lavender			Hermosa	229	259	....	....
				N.-wh. lav.				
				White	57	18	1105	....
								104.93

TABLE 3—(Continued)

32	Magenta	Magenta	*LlHhPrp*	Magenta	3177	3051	....	....
				Purple	981	1017	....	....
				Red	1011	1017	....	....
				Pink	293	339	....	....
	White			Hermosa-lav. / Hermosa / N.-wh. lav.	1662	1695	....	....
				White	108	113	7232	....
								13.68
33	Magenta	Magenta	*LlHhPrPg*	Magenta	224	220	....	....
				Purple	68	73	....	....
				Red	95	73	....	....
				Pink	25	25	....	....
	Nearly-white pink			Rose-lav. / Lavender	84	98	....	....
				Rose	18	24	....	....
				N.-wh. pink	7	8	521	....
								10.67
34	Lavender	Magenta	*LlHhPrPg*	Magenta	476	467	....	....
				Purple	138	155	....	....
				Red	158	155	....	....
				Pink	69	52	....	....
	Red			Rose-lav. / Lavender	198	207	....	....
				Rose	46	52	....	....
				N.-wh. pink	20	17	1105	....
								9.26
35	Rose	Magenta	*LlHhPrPg'*	Magenta	3787	3759	....	....
				Purple	1323	1252	....	....
				Red	1259	1252	....	....
				Pink	397	418	....	....
	Purple			Rose-lav. / Lavender	1593	1669	....	....
				Rose	439	418	....	....
				N.-wh. pink	110	140	8908	....
								16.27

Mauve. A number of our crosses make clear the relation between nearly-white lavender and white, the simplest being presented as Cross 2 (Table 1). To complete the record, it may be said that the immediate source of the nearly-white lavender plant, listed "by courtesy" in the "Description of F_1" column of the table, was a backcross of a heterozygous lavender (see below) with white. The progeny of this nearly-white lavender were scored in only two classes but the nearly-white lavender phenotype is far from uniform, varying from the White-Pale Mauve tint to flowers so lightly tinged as to be easily mistaken for white. Indeed, the separation of these from white can be done only by a person with keen color vision, working in favorable light and comparing the massed petals directly with those of known pure white strains. The numbers obtained, 261:78,

indicate clearly a monohybrid ratio. The gene pair is designated *L*, *l*. There is no dominance.

Hermosa vs. *White;* H, h

"Hermosa" is a flower color in which the petals are a pale pink lying in RIDGWAY's colors between Hermosa Pink and White. The spurred sepal is white and, like the median petal or standard, is more or less marked with chlorophyll green. A hermosa plant was crossed with a white plant, yielding four hermosa and two white offspring, thus indicating the hermosa plant to be heterozygous. One of the hermosa offspring was self-pollinated and gave hermosa and white in a ratio of 3:1 (Cross 3, Table 1). The gene concerned is designated *H*. Heterozygous hermosas (*Hh*) are, in general, paler than *HH* plants but the distinction is not sufficiently clear for scoring the hermosa plants in two groups. Dominance of *H* is, then, incomplete.

Hermosa-lavender; LH: Crosses from which come combinations of *L* and *H* show that these two genes produce a highly variable pale hermosa-lavender. We have been unable to separate phenotypically the F_2 classes due to these genes and their combinations. Each genotype, from a homozygous hermosa-lavender (*HHLL*) to white (*hhll*), is, doubtless, slightly different from every other but the gradations of color are too fine to be scored.

Nearly-white Pink vs. *White;* P^g, $P^{g'}$, $P^{g''}$, p

Plants with flowers having spurs of approximately Hermosa Pink and having petals of very pale pink color, too faint to be classified, are referred to as "nearly-white pink." When crossed with white the F_1 is even paler than the homozygous nearly-white pink. In the F_2, whites can be separated, in most cases with assurance, from the nearly-white pinks and, among the latter, homozygous and heterozygous types can be distinguished from each other with varying success. The ratio is three nearly-white pink to one white (Cross 4, Table 1), indicating a one gene difference. The genes concerned are designated P^g and *p*.

Rose; HP^g *or* $HP^{g'}$: Nearly-white pink (*hhP*^{g}*P*^{g}) was crossed with hermosa (*HHpp*). The F_1 was rose, paler than certain pure-breeding stocks, but no seed were obtained. However, these pure stocks approximating RIDGWAY's Begonia Rose and referred to by us as "rose," yield hermosa, nearly-white pink and white (Cross 15, Table 2). Homozygous nearly-white pinks extracted from Cross 15 are deeper in color than the homozygous nearly-white pink parental strain of Cross 4, (Table 1). We designate the gene of the deeper strain as $P^{g'}$ to distinguish it from P^g of the paler one. The rose color is due to a combination of genes *H* and P^g or $P^{g'}$. Included in Cross 15 (Table 2), are the progeny of one F_1 plant which was heterozygous also for noncream *vs.* cream. The progeny of this triply heterozygous plant (Table 3, Cross 15a) shows independent assortment for the three loci involved, *H*, *P*, and *W*.

Rose (*HHP*$^{g'}$*P*$^{g'}$) crossed with nearly-white pink (*hhP*^{g}*P*^{g}) gives an F_1 of rose as deep as the rose parent. The F_2 segregates into three rose: one nearly-

white pink (Cross 5, Table 1). Both rose and nearly-white pink plants of this F_2 show variation in intensity of color such as would be expected in view of the explanation given above.

Lavender; LP^{s}: When nearly-white pink (*llP^{s}P^{s}*) is crossed with nearly-white lavender (*LLpp*), the F_1 has flowers of Pale Amparo Purple-White. The F_2 yields four classes in a ratio of 9:3:3:1 (Cross 16, Table 2). In the largest class are found plants showing the F_1 color and, also, a type approximating Light Phlox Purple. The latter is precisely like homozygous strains which we have designated "lavender." The F_1 type we refer to as "pale lavender." Lavender by white (Cross 17, Table 2) involves the same genes as Cross 16 and gives essentially the same results in F_1 and F_2. It will be noticed that the deviations in the nearly-white lavender and white classes are high but complementary. This cross was made early in our experience and the large deviations are readily explained as due to classifying some nearly-white lavenders as whites. Lavender by nearly-white pink (Cross 6, Table 1) gives in F_1 a lavender as deep as the lavender parent, and in F_2 a 3:1 segregation of the parental types. In Cross 18 (Table 2) a deeper colored nearly-white pink (*llP$^{s'}$P$^{s'}$*) was substituted for the *llP^{s}P^{s}* of Cross 16. This F_1 was lavender like a homozygous strain, *LLP^{s}P^{s}*, and the F_2 included some deeper lavenders which were not separately scored but were doubtless *P$^{s'}$P$^{s'}$*. It is evident that *P$^{s'}$*, *P^{s}*, and *p* constitute a series of multiple alleles. A fourth allele in this series appears in a third and still more intensely colored nearly-white pink strain. This strain also was crossed with nearly-white lavender (Cross 19, Table 2). The F_1 was a deep lavender of approximately the intensity of the deepest lavenders of the F_2 of Cross 18. In the F_2 segregations, among the lavenders were some plants with flowers of True Purple. The extracted nearly-white pink class was like the parental type. The classes are very distinct except for the nearly-white lavenders and white. We designate the nearly-white pink gene of this strain as *P$^{s''}$*. It may be noted that the F_2 ratio suggests linkage but this interpretation does not hold under the circumstances nor is any other explanation obvious.

Returning to Cross 15 (Table 2), rose by white, variations were found in some of the F_2 color classes recorded. The nearly-white pinks fell into two fairly distinct groups, later extracted in homozygous types which were readily distinguishable. In populations including homozygous and heterozygous plants, the different genotypes cannot be distinguished consistently. Perhaps the rose parent is heterozygous, carrying two very slightly different alleles at the *P$^{s'}$* locus. Though two different nearly-white pink lines are separated, both are included under our designation *P$^{s'}$*. Also, in the rose class of that F_2, plants were found with flowers more intense in color than the parental strain. Age of flower, the added intensity resulting from aggregation of petals in doubles, and perhaps other conditions, so affected the depth of color that it was exceedingly difficult to divide this rose class with assurance. In numerous such crosses the same impression of different degrees of color has been obtained. The attempts to secure a pure stock of the deeper rose have not been successful.

Rose-Lavender; LHP^{g} *or* LHP$^{g'}$: The F_1 of lavender by rose (Cross 20, Table 2) is Phlox Purple, designated "rose-lavender." The F_2 population was exceedingly difficult to classify. It was impossible to distinguish consistently rose-lavender and lavender; therefore, these classes were combined. Rose was also difficult to separate from the combined class. The numbers 836:266:71 represent an approximation to the 12:3:1 theory satisfactory in view of these recognized difficulties.

Green-stemmed plants

The term "green-stemmed plants" is used to refer to plants having green lower stems. White plants and those bearing only the color genes *H*, *L*, and P^{g} have entirely green stems. $P^{g'}$ sometimes produces a reddish tinge on the tips of the branches and $P^{g''}$ produces a distinctly reddish color on upper stems. Lavender and rose plants which carry P^{g} have green stems below and above, but when these have $P^{g'}$ they have reddish color on upper branches only. When $P^{g''}$ was used in synthesizing lavender, the plants of the lavender and nearly-white pink classes showed even more color on the upper stems. This would doubtless also be the case if $P^{g''}$ were used to synthesize rose.

Pink vs. *Nearly-white Pink, or White;* P^{r}, P^{g}, p

Our strains include a number with stems deeply colored, at least up to the first node, and bearing flowers with pink petals, the spurs being considerably deeper in color than in the strains bearing P^{g}, $P^{g'}$ or $P^{g''}$. Different strains vary in the extent of color on the stems. In some strains, it is restricted to below the second node; in others, it continues to the tips of the branches and onto the midribs of the leaves. Different homozygous strains are highly constant in the distribution of color on the stems and in the intensity of color on stems, petals, and spurs. The intensity of color on the petals is not only quite constant within a strain, but in different strains varies much less than the color on the spurs and stems. The color of the petals in different strains ranges from Deep Rose Pink to La France Pink; spur-color ranges from Rosolane Purple to Rose Color. In plants with the darkest flowers the stems are such an intense red as to appear almost black at the base, and even the tips of the branches are as dark as Victoria Lake. Strains with palest flowers have stems with a maroon tinge at the base; at the tip they are of almost the same color as in the green-stemmed plants. For convenience, we call all of the strains "pink." The strain most commonly used in crosses has petals Eosin Pink, spurs Rose Color, stems intermediate between the two extremes described above. The F_2's of two crosses of pink by white are summarized under Cross 7 (Table 1), showing a ratio of 3 pink:1 white. The distinction between these classes is perfectly definite. The factor concerned is designated P^{r}, the superscript *r* referring to the red color on the lower stem in contrast with the green lower stems produced by P^{g}, $P^{g'}$, and $P^{g''}$.

In one case, a true-breeding pink crossed with a white, gave five F_1's which yielded, in the F_2, 3:1 ratios which are included under Cross 7 (Table 1); but

also, one F_1 which when selfed produced 710 red-stemmed: 12 green-stemmed plants (included in Cross 7a). Since there is perfect correlation between stem and flower color, a portion of the F_2 plants was scored when not in bloom. Eight pinks of this F_2 were self-pollinated; they gave red stems and green stems in the following numbers: 94:1, 172:2, 101:2, 193:0, 17:0, 66:0, 295:0, and 166:7. The first three of these approximate a 63:1 ratio and, combined with the F_2, yielded the 1077 pinks:17 whites. The next four of these F_2's apparently bred true although, in at least two cases, the numbers are so small that a 63:1 ratio is not excluded. In the last of these F_2's the numbers are nearer a 15:1 ratio than to 63:1 or 3:1. The 63:1 ratio may be explained as the result of triple identical factor pairs with dominance in each pair and with independent assortment among them. If an entire typical set of the 63 group were tested, the expectation may be compared with our observed numbers as follows:

	To breed true	To give 63:1	To give 15:1	To give 3:1
Observed numbers	4	3	1	0
Observed proportion	32	24	8	0
Expectation	37	8	12	6

The 63:1 and 15:1 ratios occurred in the progeny of only one F_1 plant. None was produced beyond the F_3 and the stock has now been lost. The cross has been repeated but the exceptional results have not recurred. Had cytological tests of the plants which yielded the ratios of 63:1 and 15:1 been made, they might have provided a basis for explaining the exceptional ratios.

Material for a further analysis of the relation of P^r is provided by a cross of pink with nearly-white pink (Cross 8, Table 1). The F_2 ratio of 3 pinks:1 nearly-white pink shows that P^r and P^g are alleles. The pinks of the F_1 and the F_2 are phenotypically uniform in floral color. P^r, then, is an addition to the series of alleles at the *P* locus and is dominant to P^g and *p*.

Purple; LP^r: The genes *L*, *H* and P^r are concerned in the deeper colors, purple, red and magenta.

When pink is crossed with nearly-white lavender the F_1 is "purple," RIDGWAY's True Purple. The F_2 segregates into the classes and in the ratio to be expected if purple is caused by genes *L* and P^r. Details are shown under Cross 21 (Table 2). Crosses 21a and 21b are like Cross 21 but involve pink parents successively deeper in color. The genes of these deeper colored strains are styled $P^{r'}$ and $P^{r''}$

A cross in which P^g is involved instead of *p* is presented as Cross 22 (Table 2). This is lavender by pink. The F_1 is purple, as expected. The F_2 gives a satisfactory ratio in the classes expected on a basis of an allelic relation between P^r and P^g. Cross 22a (Table 3) is the same as Cross 22 except that it takes into account the noncream, cream factor. A cross of purple by white (Cross 23, Table 2) involves the same factors as in Cross 21 and gives the same classes and ratio in the F_2. A similar F_2 is given under Cross 24 (Table 2). This F_2 was scored late in the season when the distinction between nearly-white lavender and white was ob-

scured. This situation made it necessary to combine these two classes and to compare with a 9:3:4 ratio.

Purple crossed with pink (Cross 9, Table 1) gives the expected classes and ratio in F_2. Cross 10 (Table 1) shows the results of crossing purple with lavender; Cross 25 (Table 2), the results of a cross of purple with nearly-white pink. The F_2 monohybrid ratio in Cross 9 and the dihybrid ratio in Cross 25 further confirm the conclusion that P^r and P^s are allelic to each other.

The various crosses involving purple, here referred to, have indicated no linkage among the genes concerned, i.e. those at loci *L*, *P*, and *W*.

Faded Purple: Several years ago there appeared in a strain of purples, previously breeding true, a plant with stems red like the purple parent with flowers intermediate between RIDGWAY's Light Phlox Purple and Phlox Purple. Both petals and spurs bear this color; but in the petals it is irregularly distributed, giving a faded appearance. Except for the color on the stem the total effect is very similar to the rose-lavender previously described. This flower color is designated "faded purple." Its genic analysis is not yet complete.

Red; HP^r: Flowers matching RIDGWAY's Spectrum Red are referred to as "red." Crosses 11 and 12 (Table 1), and Cross 26 (Table 2), involve red and show that it is produced by the combination of *H* and P^r. Crosses 27, 27a and 27b (Table 2) give F_2 data on crosses involving allelic genes for the deeper colors of the P^r type. Different intensities of stem colors in corresponding classes of these sets are noticeable, especially when $P^{r'''}$ is involved. In Cross 27a there is a marked excess of reds and in Cross 27b a marked deficiency in the rose plants as compared with expected numbers. In spite of these irregularities, the results show that red possesses gene *H*, and P^r in one of its grades of intensity. The various crosses involving red make it clear that there is no linkage between the loci concerned, *H* and *P*.

Magenta; LHP^r: "Magenta" flowers are approximately RIDGWAY's Tyrian Rose. Crosses showing magenta in the F_1's are set forth in Table 1, Crosses 13 and 14; Table 2, Crosses 28, 29, and 30, and in Table 3, Crosses 31, 32, 33, 34, and 35. In Crosses 31 and 32 (Table 3), difficulty in distinguishing nearly-white lavender, hermosa-lavender and hermosa has made it necessary to combine these classes; in the presence of hermosa and hermosa-lavender plants the separation of whites from nearly-white lavenders is also uncertain. Similar difficulties have been encountered in Crosses 33, 34, and 35 (Table 3), in distinguishing rose-lavender and lavender. It is abundantly clear from these crosses that magenta bears genes *L*, *H*, and P^r and that there is independent assortment between them.

Red-stemmed plants

The stems of purple, red, and magenta plants bear red pigment, at least up to the first node, and, in many strains, the entire stems are intensely red. The range of stem colors is the same as the range in the pink strains. The factor common to all these is P^r, showing that P^r is the gene responsible for the red color on the lower stem.

Unscorched vs. *scorched;* Sc, sc

A packet of seed, obtained in 1925 from Haage and Schmidt and designated by them "Kupferrot," yielded four dwarf plants. Two of them bore typical magenta flowers; and two, flowers of approximately Pomegranate Purple, a type new to us. The edges of the petals tend toward Burnt Lake and are curled inward giving an impression which is suggested in our name for the trait, "scorched." Scorched magenta crossed with red gave unscorched magenta in F_1; and, in F_2, unscorched magenta: unscorched red: scorched magenta: scorched red in a ratio of 9:3:3:1 (Cross 36, Table 4). The recessive gene for scorching is referred to as *sc*, the dominant allele as *Sc*. Scorched magenta crossed with white (Cross 32a, Table 4) gave unscorched magenta in F_1. In the F_2, scorched appeared only in the magenta and red colors. Since the *sc sc* gene did not express themselves in any except red and magenta classes and since three pale colors had to be combined as usual, the expected ratio is 81:27:27:9:36:12:60:4, instead of the one

TABLE 4

Crosses involving Scorched

Number of cross	Description of parents	Description of F_1	F_1 genotype	F_2 Phenotypes: Colors	Obs.	Calc.	Tot.	χ^2
26a	Scorched	Unscorched	*HhP^{r}p-*	Uns. Red	1130	1081	....	....
(Cf. 26,	Red	Red	*Scsc*	Sc. Red	289	360	....	....
Table 2)				Pink	523	480	....	....
	Unscorched			Hermosa	449	480	....	....
	White			White	170	160	2561	....
								22.70
32a	Scorched	Unscorched	*LlHh-*	Uns. Mag.	2359	2288	....	....
(Cf. 32,	Magenta	Magenta	*P^{r}pScsc*	Sc. Mag.	818	763	....	....
Table 3)				Uns. Red	764	763	....	....
				Sc. Red	247	254	....	....
	Unscorched			Purple	981	1017	....	....
	White			Pink	293	339	....	....
				Hermosa-lav. / Hermosa / N.-wh. lav.	1662	1695	....	....
				White	108	113	7232	....
								14.73
32b	Scorched, noncream	Unscorched, noncream	*WwScsc*	Uns. n-cr.	611	562	....	....
	Unscorched, cream			Uns. cream	150	188	....	....
				Sc. n-cr.	181	188	....	....
				Sc. cream	58	62	1000	....
								12.49
36	Scorched Magenta	Unscorched Magenta	*LlHH* *P^{r}P^{r}Scsc*	Uns. Mag.	418	419	....	....
				Sc. Mag.	133	140	....	....
	Unscorched Red			Uns. Red	146	140	....	....
				Sc. Red	49	47	746	....
								0.70

typical of a tetrahybrid segregation. A scorched red of the F_2 of cross 32a (Table 4) was self-pollinated, establishing a homozygous strain. A plant of this strain crossed with white (Cross 26a, Table 4), gave unscorched red plants which yielded an F_2 in which scorched appeared only in red plants. Under these circumstances the expected ratio is 27:9:12:12:4, instead of the usual trihybrid ratio. A scorched red of this F_2 was selfed, yielding 14 scorched reds and one unscorched hermosa. This phenotypically unscorched hermosa was then crossed with two of its scorched red sibs. With one, it yielded eight scorched reds; with the other, 11 scorched reds and three hermosas that showed no sign of the scorched trait. The 19 scorched red plants in the progeny of these two crosses can be explained only on the basis that the hermosa parent carried the recessive genes *sc sc*. It may be presumed that colors other than hermosa, which do not show the scorched trait, may nevertheless carry the *sc sc* genes. The *sc sc* genes express themselves in combination with genes LHP^r and $llHP^r$ but do not express themselves in combination with *LH* nor LP^r, neither with *L*, *H*, P^r, or *W* as the sole color producing genes, or with the complete recessive cream. No crosses have been made that would determine whether the scorched trait can be exhibited by genotypes bearing P^s.

Data of the F_2 progeny of one of the F_1's of Cross 32a are set up to reveal any linkage between *Sc* and *W* (Cross 32b, Table 4). Crosses 32a, 32b, and 36 make it clear that the locus *Sc* is not in chromosomes with *L* nor *W*.

DISCUSSION

An attempt at correlating with ours the findings of other investigators of floral colors in the garden balsam, is presented in Table 5.

KANNA (1934) shows clearly a single factor difference between red and pink and another single factor difference between purple and pink. Also, he shows that the combination of factors for red and purple gives magenta in the F_1; and, in the F_2, magenta, purple, red and pink in the ratio of 9:3:3:1 as expected. Our findings are in agreement with this.

But our genic analyses of pink and the pale colors are quite different from KANNA's. He finds grounds, in his records on crosses of deeply colored plants with pale colors and white, for assigning three linked genes (*D*, *W*, and *S*) to each of the four red-stemmed color classes (pink, red, purple, and magenta). These genes collectively, then, correspond with our P^r gene. An examination should be made of the justification for KANNA's genes *D*, *W*, and *S*. The difference between green-stemmed plants and red-stemmed plants he ascribes to a factor *S*. This gene produces red on stems and more intense color in flowers, as contrasted with *s* for green stems and dilute color of petals. For pure white, he considers a gene *w* essential; *W*, in absence of other color genes, gives a dilute pink or very dilute red. He maintains that *W* and *S* are linked, and estimates the crossovers between them at 1.4 percent. The estimate of 1.4 percent in this specific case is based upon the results of self-pollinating a heterozygous pink: 208 pinks, 64 whites, and 2 dilute pinks. This is comparable with our Cross 7 (Table 1)

TABLE 5

Colors and genotypes of KANNA (1934), BEDELL, HAGIWARA *and* RASMUSON *correlated with this paper*

DAVIS, TAYLOR, ASH		KANNA					
Name	RIDGWAY	Name	RIDGWAY	BEDELL	HAGIWARA	RASMUSON
White *llhhpp*	.....	White, with white spur *rr(psp)(psp)(dws)(dws)*	.....	White *mp*	White pm & *Rc*, *rC*, or *rc*	White
Nearly-white lavender *L*	White-Pale Mauve	Very dilute purple *RW*	Pale Vinaceous Lilac	Classed as white *Mp*	Classed as white *PC*	Classed as white (?)
Hermosa *H*	Hermosa Pink-White	Very dilute red *PW* Red so dilute as to be indistinguishable from white *P* Very dilute red with white spur *W*	Hermosa Pink	Classed as white *Rp*	Classed as white *MC*	Classed as white (?)
Hermosa-lavender *LH*	.....	Very dilute magenta classed as very dilute purple *RPW*, *RP*	.....	.....	Classed as white *MPC*	Classed as white (?)
Nearly-white Pink *P*ᴇ, *P*ᴇ′ or *P*ᴇ″	Petals nearly white, tinged with pink, spurs are Hermosa Pink	Very dilute pink (*S*ᵖ?) *W* White, with colored spur *P*S*ᵖ or *S*ᵖ Very dilute red with colored spur *S*ᵖ*W* Dilute pink *W* White *S*ᵖ (?)	Shell Pink	.....	.....	.....
Rose *HP*ᴇ, *HP*ᴇ′ or *HP*ᴇ″	Begonia Rose	Dilute Red *P(DW)*	Begonia Rose Eosin Pink	.....	.....	.....
Lavender *LP*ᴇ, *LP*ᴇ′ or *LP*ᴇ″	Light Phlox Purple	Dilute Purple *R(DW)*	Rose-Purple	.....	.....	.....

TABLE 5—(Continued)

Colors and genotypes of KANNA (1934), BEDELL, HAGIWARA *and* RASMUSON *correlated with this paper*

DAVIS, TAYLOR, ASH		KANNA					
Name	RIDGWAY	Name		RIDGWAY	BEDELL	HAGIWARA	RASMUSON
Rose-lavender *LHP^{g}* or *LHP$^{g'}$*	Phlox Purple	Dilute magenta, indistinguishable from dilute purple and classed with it	*RP(DW)*	Not described	.....	.....	.....
Pink *P^{r}*, *P$^{r'}$* or *P$^{r''}$*	Petals are Eosin Pink spur is Rose Color	Pink	*S^{p} (?)(DWS)*	Geranium Pink LaFrance Pink	Peach Blossom Pink *P*	Peach *pmRC*	"Rosa" *A*
Red *HP^{r}*	Spectrum Red	Red	*P(DWS)*	Spectrum Red	Rosy-Scarlet *RP*	Red *MRC*	"Rot" *RA*
Purple *LP^{r}*	True Purple	Purple	*R(DWS)*	Rhodamine Purple	Magenta-mauve *MP*	Purple *PRC*	"Blau" BA
Magenta *LHP^{r}*	Tyrian Rose	Magenta	*RP(DWS)*	Tyrian Rose	Magenta *MRP*	Red-purple *PMRC*	"Blaurot" *BRA*

* Was *R* in original paper, obviously a misprint.
In the first line of the body of the table are represented all genes of which contrasting alleles produce color.
On lines below the first only the contrasting alleles which produce color are represented.

which gave 1476 pinks, 450 whites, and no nearly-white pinks. In view of the numerous progeny of our cross, it is our judgment that the two dilute pinks do not warrant the conclusion that there are two linked genes involved. Certainly in our cases the single gene, which we have designated P^r, explains not only the difference in floral and stem colors between the pinks and the whites, which KANNA ascribed to linked genes *W* and *S*, but also the difference between red and dilute red (our rose), which he ascribed to *S* alone.

The basis for KANNA's dilution gene *d* is more difficult to analyze. His Table XLVII, upon which he relies to show the existence of this gene, is so nearly a repetition of his Table XLV that one suspects a pertinent table is omitted and this one substituted by mistake. As it stands, no adequate evidence is presented for his statement that, in the cross of magenta by white, the very dilute color of certain flowers "is due to the effect of a diluting gene *d*, the common flowers carrying its dominant mate" and that "close or almost complete linkage takes place between *d* and *w*." The appearance of very dilute flowers in the numbers he records may be adequately explained as the progeny of a magenta of the genotype $LlHhP^rp$ as shown in our Crosses 31 and 32 (Table 3). The very dilute flowers would then be a composite of our hermosa-lavender, hermosa, nearly-white lavender, and white. It is our opinion that satisfactory evidence for closely linked genes *D*, *W*, and *S* is lacking. On the other hand, the color differences which KANNA ascribes to them seem to us identical with those which we have shown to be due to the allelic series *P*.

Having seriously neglected spur-color in most of his descriptions, in a cross represented in his Table L in connection only with green-stemmed plants, KANNA assumes a special gene, S^p, for color of spurs. The ratio he presents is quite inadequate evidence of linkage between *P* and S^p for which he estimated crossovers of seven percent. We have not found it necessary to assume a special gene for spur-color since, as shown above, various grades of spur-color in green-stemmed plants are dependent on P^g in its various intensities.

Our identification and interpretation of KANNA's genotypes and phenotypes are set forth in Table 5. The arrangement is designed for comparison with our classes. On the assumption that his *R*, *P*, and (*DWS*) are respectively equivalent to our *L*, *H*, and P^r, his genotypes and ours for magenta, purple, red, and pink are in harmony. Our rose-lavender, which we cannot distinguish from lavender, corresponds with KANNA's dilute magenta ("magenta . . . in the green-stemmed variety") which he classes with dilute purple. Rose-lavender and lavender differ from magenta and purple only by our gene difference P^g, P^r or by KANNA's *s*, *S*. The difference between our rose and KANNA's dilute red on the one hand, and red on the other, is due to the same gene. Our hermosa-lavender is not consistently distinguishable from nearly-white lavender and KANNA's corresponding genotypes are also indistinguishable. The genotypic differences between hermosa-lavender and nearly-white lavender on the one hand, and magenta and purple on the other, are, in our terminology, due to the lack of both P^g and P^r. In KANNA's terminology the differences are due to the lack of *D* and *S*. In his very dilute

magenta *W* also may be lacking. To correspond with our hermosa, which contains the factor distinguishing red from pink, we have gathered certain of KANNA's phenotypic classes which are described in nearly identical terms, but to which he leads us to assign different genotypes. His very dilute red is represented as containing *P* and *W*, red so dilute as to be indistinguishable from white as having *P* but not *W*, and very dilute red with white spur as having *W* but not *P*. We venture to suggest that any of these crossed with pink would give identical results, whereas, according to KANNA's genotypes, the first two should give red and the last pink. Suitable crosses may be devised to distinguish further among his genotypes which we have grouped under our hermosa-lavender. Our crosses have given us no reason to think that our hermosa or our hermosa-lavender groups are complex except that the *H* gene in the one and the *H* and *L* in the other may be homozygous or heterozygous.

Further difficulties in our interpretation of his work appear in connection with the nearly-white pink group, and arise out of KANNA's disregard of stem-colors and spur-colors in many of his descriptions. Apparently, he considers that *W* may give phenotypically either very dilute pink (with S^p?), very dilute red with colored spur (with S^p), or dilute pink. A plant bearing *S*, which gives red lower stems, may also be referred to as dilute pink. In our experience this last belongs in the pink group (P^r). S^p (with or without *P*) gives white with colored spur but on occasion is referred to by KANNA simply as white. It appears to us that we have solved these confusions thru recognition of various P^s alleles as set forth in preceding pages. We are constrained to believe that any of KANNA's types which we have associated with our nearly-white pink, would give lavender if crossed with nearly-white lavender. So far, then, as genes dealt with in Table 5 are concerned, the white phenotype (care being exercised to exclude any plants with color on stems, spurs, or petals) includes only one genotype.

With only two exceptions KANNA's classes and ratios are explicable on the simpler hypotheses which our figures have established. One of these exceptions is the case of the two "crossover" pinks referred to above. The other exception is the case of six very dilute purple plants *RP*(*dws*) or R^p(*dws*) which are shown in his Table XLVI as derived by self-pollination from a pink *rrpp*(*DWS*)(*dws*). He makes no explanation of this clear impossibility and our scheme of genes has none to offer.

BEDELL (1927), HAGIWARA (1926) and RASMUSON (1920) apparently lacked all the phenotypes bearing the P^s alleles at the *P* locus which have added such opportunities and such obstacles in the work of KANNA and ourselves. As may be seen from Table 5, each of those investigators has solved the relations of the deeper colors. However, each has failed to distinguish essential classes in the green-stemmed plants, and has counted all of these as whites. BEDELL and HAGIWARA recognized that their white group must include a variety of genotypes. HAGIWARA introduces a chromogen factor *C* which the genetic data do not demand.

SUMMARY

1. All the flower colors of the garden balsam occur in duplicate series. Each color in the noncream series bears a dominant gene *W*, and has its counterpart in the cream series which bears the recessive genes *ww*. Of the noncream series the lowest is pure white, and cream is the lowest member of the cream series.

2. The occurrence of the following genes has been established: *L,l* for nearly-white lavender versus white, with no dominance. *H,h* for hermosa versus white, with *H* partially dominant. P^{s}, $P^{s'}$ and $P^{s''}$ versus *p*, a series of genes each producing its characteristic intensity of nearly-white pink on petals and of pink on spurs in contrast with the white of *p*. P^{s}, $P^{s'}$, $P^{s''}$ plants have green lower stems. P^{r}, $P^{r'}$, and $P^{r''}$, as additional alleles of *p*, each producing, not only its own intensity of pink color on petals and spurs, but also, its own characteristic red on the stems in contrast with *p* and the P^{s} series.

3. The genic composition of complex colors has been established as follows:
Green-stemmed plants:—*LH*, hermosa-lavender; HP^{s}, $HP^{s'}$, or $HP^{s''}$, rose; LP^{s}, $LP^{s'}$, or $LP^{s''}$, lavender; LHP^{s}, or $LHP^{s'}$, rose-lavender.
Red-stemmed plants:—HP^{r}, $HP^{r'}$, or $HP^{r''}$, red; LP^{r}, $LP^{r'}$, or $LP^{r''}$, purple; LHP^{r}, magenta.

4. An analysis of the genetic constitution of faded purple has not yet been completed.

5. A recessive gene for scorched (*sc*) has been shown to affect the color of reds and magentas. This gene can be carried in the homozygous state without expressing itself phenotypically. It has been shown to be so carried by hermosa.

6. It has been shown that the genes of loci *W*, *L*, *H*, and *P* assort independently, and that *Sc* is not linked with the *L* nor with the *W* locus.

7. Based upon the genes we have established, we have presented reinterpretations of the data of other investigators.

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LITERATURE CITED

BEDELL, B. S., 1927 The inheritance of certain characters in *Impatiens balsamina* Linn. Gard. Chron. **82**: 349–351.

DAVIS, DONALD W., 1927 Inheritance of flower colors associated with green stem-color in the Garden Balsam. Anat. Record (2) **37**: 179.

1932 Impatiens. Proc. 6th Intern. Congr. Genet. **2**: 306–310.

DAVIS, D. W., LUCY ANN TAYLOR and R. P. ASH, 1939 The genes determining floral colors in *Impatiens balsamina* L. Proc. Va. Acad. Sci. 1938–39. 32.

HAGIWARA, TOKIO, 1926 Genetic studies in balsam. I. Bot. Mag. (Tokyo) (473) **40**: 295–306.

KANNA, BENSO, 1926 On the inheritance of balsam. Bot. Mag. (Tokyo) (479) **40**: 599–619.

1934 Genetic studies in *Impatiens balsamina* L. J. Coll. Agric. Tokyo Imp. Univ. (3) **12**: 421–477.

RASMUSON, H., 1920 Die Hauptergebnisse von einigen genetischen Versuchen mit verschiedenen Formen von Tropaeolum, Clarkia, und Impatiens. Hereditas **1**: 270–276.

RIDGWAY, ROBERT, 1912 *Color Standards and Color Nomenclature.* p. i–iii, 1–44, pl. I–LIII. Pub. by the author. Washington, D. C.

CHEMICAL ASPECTS OF THE INHERITANCE OF FLOWER COLOR IN IMPATIENS BALSAMINA L.

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RECENT applications of chromatographic techniques (BATE-SMITH 1948, 1949) have facilitated identification of anthocyanin pigments and related compounds in micro quantities, thus permitting extension of the combined genetic and biochemical work initiated by SCOTT-MONCRIEFF (1931) and extended by many others. The genetic studies by the late D. W. DAVIS and his associates (1958) on the inheritance of flower color in *Impatiens balsamina* provide a material which seems particularly well suited for such studies. The balsams have a short life cycle, large flowers, and rather precise differentiation of color classes. Various flower colors exhibited in this species have been ascribed previously to the presence of glycosides of three anthocyanidins: pelargonidin, peonidin and malvidin, (BEALE *et al.* 1941; HAYASHI *et al.* 1953; FORSYTH and SIMMONDS 1954) but no association of the pigment content with the genetic structure of the plant was made in these studies. The species is one of a family of herbaceous plants which is known to produce leucoanthocyanins (BATE-SMITH 1954) and flavonols (HAYASHI *et al.* 1953); hence an intensive genetical and biochemical study may reveal the metabolic interrelations of several classes of similar compounds.

This paper deals with the correlation between genotype and complement of both anthocyanins and leucoanthocyanins. The data are still limited but allow some conclusions as to stages in the biosynthesis of the anthocyanins.

MATERIALS AND METHODS

Genetic material: One of the authors (R.A.) enjoyed the privilege of working with PROFESSOR DAVIS for several years preceding his death and was able to preserve stocks from which all color types were regained. All plants analyzed were of the noncream series, *i.e.*, *WW*, and only one of each of the several alleles designated P^r or P^s by DAVIS (1958) was employed in the biochemical studies.

Twelve potential phenotypes are recognized as consequence of interaction of the genes *L*, *l*, *H*, *h*, P^r, P^s, and *p*, although some, especially *L-H-*P^s and *L-hh-*P^s, can scarcely be distinguished without knowledge of the genetic history. In certain of the paler color classes, there is an obvious quantitative difference between the heterozygote and the dominant homozygote, although no qualitative differences

* A portion of the work included in this paper was submitted as part of a thesis by R. E. ALSTON in partial fulfilment of the requirements for the Ph.D. degree, Department of Botany, Indiana University.

in pigment complement have been detected which can be ascribed to gene dosage. In subsequent portions of this paper the dominant homozygote and the heterozygote will be considered equivalent and genotype will be indicated by a single symbol for each locus.

A breeding program is in progress which should ultimately yield isogenic stocks, but analyses presented here were performed on less highly refined material. With few exceptions the pigment complements have been confirmed on segregants of crosses between multiple heterozygotes and an inbred white stock. The exceptions include those genotypes which involve *Pt* in association with other dominant genes.

Methods of pigment analysis: Three separate methods of pigment extraction have been employed at various stages in the qualitative analysis of the pigments present in the various phenotypes. Since the principal complication of the present study lies in the distinction between derivatives of leucoanthocyanins and those of anthocyanins, each of the methods is described below.

In initial determinations the desired tissue, either fresh or frozen, was boiled for five to ten minutes in one percent HCl, then filtered. An equal volume of concentrated HCl was added to the filtrate and the mixture boiled again for five to 15 minutes. After another filtration, the acid solutions were exhaustively extracted with ether to remove flavonol and other substances which complicated the chromatograms. (The ether extraction was omitted in later assays.) The anthocyanidins were partitioned into a small volume of isoamyl alcohol and applied to chromatograms. These amyl alcohol solutions contained mixtures of anthocyanidins derived from conversion of leucoanthocyanins as well as from hydrolysis of anthocyanins.

Preliminary tests revealed that repeated extraction by boiling with small quantities of 0.04 percent HCl in 95 percent ethanol without maceration of the flower parts would remove only traces of leucoanthocyanins from white flowers yet would completely remove visible pigments from intensely colored flowers. The residual tissue contained considerable leucoanthocyanin which could be converted with unknown efficiency to anthocyanidin and extracted by boiling with 50 percent HCl. These anthocyanidins were partitioned into isoamyl alcohol and applied to chromatograms in determining the class of leucoanthocyanins present in the tissue. The ethanolic extracts, which contained the anthocyanins, were evaporated to near dryness, then hydrolyzed with 50 percent HCl and partitioned into isoamyl alcohol. The aglycones revealed in these latter extracts by chromatography were not entirely reliable as indicators of the anthocyanins present since traces of anthocyanidin were obtained from completely white flowers.

To assay the anthocyanidins present as visible derivatives, the tissues were extracted with one percent HCl in 95 percent ethanol. The extracts were concentrated over a steam table, then applied directly as long streaks and developed in the t-butanol or ethyl acetate solvents (see below). The center of each anthocyanin band was cut from the chromatogram and the pigments eluted from the

paper by allowing one percent HCl in 95 percent ethanol to flow the length of the strip. The eluates were concentrated over steam, hydrolyzed, partitioned into isoamyl alcohol and applied as a solution of the aglycones. Since preliminary tests had shown that at least the bulk of the leucoanthocyanin was immobile in the t-butanol solvent, aglycones determined in this fashion were considered to be derived from anthocyanins. This method has been successful only with the stronger bands of the more intensely colored forms. Anthocyanins of the more weakly pigmented phenotypes have been identified by comparison on the same chromatogram with anthocyanins from the more intensely pigmented flowers.

Pigments were applied as short streaks to Whatman #1 filter paper from isoamyl alcohol or from one percent HCl in 95 percent ethanol depending on the extraction method. The chromatographic technique was essentially the ascending method described by WILLIAMS and KIRBY (1948). Three solvent mixtures were employed for anthocyanidin identification. A mixture of t-butanol, acetic acid, and water (3:1:1) forms a single phase and provides moderate resolution of both anthocyanins and anthocyanidins. Where several anthocyanidins are present in the same extract, however, overlapping may occur, hence other solvents or diagnostic color tests must be employed simultaneously. The Forestal solvent described by BATE-SMITH (1954) gives excellent resolution, while a mixture of m-cresol, acetic acid, and water (55:7:6) accelerates the methylated pigments and permits identification where complex mixtures of pigments occur. Representative Rf values for the principal anthocyanidins in each of these solvents are listed in Table 1.

TABLE 1

Average Rf values of anthocyanidins in three solvent mixtures

	t-butanol: acetic acid: water (3:1:1)	m-cresol: acetic acid: water (55:7:6)	acetic acid: hydrochloric acid: water (30:3:10)
pelargonidin	.64	.63	.72
peonidin	.47	.75	.73
cyanidin	.41	.33	.53
malvidin	.36	.85	.67
petunidin	.30	.48	.50
delphinidin	.18	.12	.32

Extracts of intensely colored flowers yield several anthocyanins. In order to determine the number of anthocyanins present in such extracts, a complex solvent was developed consisting of a mixture of ethyl acetate, t-butanol, acetic acid and water (5:4:1:3). This mixture sweeps flavonols, naphthoquinones, and other interfering substances to the top of the paper while providing excellent definition and separation of the derivatives of individual anthocyanidins. Derivatives of unlike anthocyanidins are moderately well separated. Thus magenta flowers (*LHP*^{r}), which yield two anthocyanidins from anthocyanins, provide five distinct bands in chromatograms of unhydrolyzed extracts. Peonidin and

cyanidin derivatives overlap in this solvent but are distinguishable by their fluorescence and their color after exposure to $AlCl_3$.

Since Rf values fluctuate with ambient conditions, all identifications were based ultimately on comparisons with anthocyanidins obtained from species which had been investigated by other authors. After considerable selection, the following were chosen as useful sources of standard anthocyanidins: *Pelargonium zonale*—pelargonidin with other pigments; *Salvia splendens*—pelargonidin; *Centaurea cyanus* (*deep blue*)—cyanidin; *Viola papilionaceae* or *Delphinium ajacis* (blue)—delphinidin; *Peonia lactiflora* (deep red)— peonidin; *Parthenocissus quinquefolia* (fruits)— malvidin; and *Petunia hybrida* var. Alderman—petunidin with malvidin. Quantities of these were frozen and extracted as needed.

While certain of the ROBINSONS' tests (1931) were employed in early stages, the only reagent used regularly was two percent $AlCl_3$ (ethanolic). When sprayed on chromatograms this accentuates color differences. Pigments containing orthohydroxyl groups (cyanidin, delphinidin, and petunidin) turn blue whereas others are relatively unaffected.

Certain genotypes control the production of flowers which are distinctly bicolored. While in some cases this can be attributed to differences in the concentration of the same pigment, in others the difference in hue suggests that the pigment complements are different. Preliminary tests of pink flowers (lhP^r) revealed real differences in the anthocyanidins present, hence in all subsequent analyses petals and sepals were examined separately. The single upper petal which is exposed in the bud and is somewhat intermediate in color was omitted from both samples.

RESULTS

Leucoanthocyanins: Anthocyanidins in extracts of buds (Table 2, column a) were derived entirely from leucoanthocyanins since care was taken to exclude any buds which had begun to show visible pigmentation. Leucoanthocyanins are present in both sepals and petals of the earliest bud stages which could be dissected (about 3 mm length) and occur also in other tissues. The buds analyzed by chromatography ranged from six to nine millimeters in length and were not separated into size classes.

Cyanidin and delphinidin were obtained from buds of all phenotypes with cyanidin generally present in excess. Variations in the relative quantity of delphinidin could not be associated with the presence or absence of any single gene. The dominant allele at the *H* locus, however, is perfectly correlated with the presence of a leucoanthocyanin which yields pelargonidin. No qualitative differences between sepals and petals of colorless buds have been detected.

Leucoanthocyanins of mature pigmented flowers were examined after prior removal of the visible pigments through alcoholic extraction. The leucopigments of mature flower parts (Table 2, column d) seem identical with those of immature buds with the one exception that delphinidin has not been obtained from the sepals of pink flowers (lhP^r). Cyanidin was abundant in these extracts hence it is possible that delphinidin was overlooked. Only traces of delphinidin were

TABLE 2

Anthocyanidins from genetic races of Impatiens balsamina *by various extraction methods*

PETALS

Genotype	Phenotype	Colorless buds	Mature flowers				Anthocyanidins from anthocyanins
		(a)	(b)	(c)	(d)	(e)	(f)
lhp	White	C,D	C,D	C	C,D	....	none
Lhp	Pale lavender	C,D	C,D,M	....	C,D	....	M
lHp	Pale pink	P,C,D	P	P	P,C,D	....	P
LHp	Pale pink-lav.	P,C,D	P,D,M	P,M	P,C,D	....	P,M
lhP^g	Pale pink	C,D	P,C	P,C	C,D	....	P
LhP^g	Lavender	C,D	M	M,C(tr.),D	C,D	....	M
lHP^g	Rose	P,C,D	P	P	P,C,D	....	P
LHP^g	Lavender	P,C,D	P,M	P,C(tr.), D(tr.),M	P,C,D	....	P,M
lhP^r	Pink	C,D	P,Peon.	P,C	C,D(tr.)	P	P,Peon.(?)
LhP^r	Purple	C,D	M	M	C,D	M	M
lHP^r	Red	P,C,D	P	P	P,C,D	P	P
LHP^r	Magenta	P,C,D	P,M	P,M	P,C,D	P,M	P,M

SEPALS

Genotype	Phenotype	Colorless buds	Mature flowers				Anthocyanidins from anthocyanins
		(a)	(b)	(c)	(d)	(e)	(f)
lhp	White	C,D	C,D	C	C,D	....	none
Lhp	White(?)	C,D	....	....	C,D	....	none(?)
lHp	White(?)	P,C,D	P,C	no yield	P,C,D	....	none(?)
LHp	White(?)	P,C,D	C,D,M	no yield	P,C,D	....	M(?)
lhP^g	Pale pink	C,D	C	C,Peon.	C,D(tr.)	....	Peon.
LhP^g	Pale lavender	C,D	M	M,C(tr.)	C,D	....	M
lHP^g	Pale pink	P,C,D	P,Peon.	P,C	P,C,D(tr.)	....	P,Peon.
LHP^g	Pale lavender	P,C,D	M	C,D(tr.), M(tr.)	P,C,D	....	M
lhP^r	Deep pink	C,D	C,Peon.	C,Peon.	C	C,Peon.	C,Peon.
LhP^r	Lavender	C,D	M	C,M	C,D(tr.)	M	M
lHP^r	Pink	P,C,D	P,Peon	P,C	P,C,D	P,C,Peon.	P,C,Peon.
LHP^r	Lavender	P,C,D	M	C(tr.),M	P,C,D	....	M

The pigments in columns (a) and (b) were extracted with aqueous one percent HCl. Pigments of column (c) were obtained through extraction with ethanolic 0.04 percent HCl, and the colorless residual tissue yielded the pigments of column (d) after boiling with 50 percent HCl. Column (e) lists the aglycones obtained by hydrolysis of anthocyanins eluted from chromatograms. Column (f) lists the anthocyanidins believed to have been recovered from visible pigments in the flowers.

Specific anthocyanidins are represented by the following symbols in the table: pelargonidin = P; cyanidin = C; delphinidin = D; malvidin = M; peonidin = Peon.

present in similar extracts from petals of the same flowers. There seems no explanation for its absence which may be directly attributed to the gene P^r since corresponding combinations involving the alleles P^g and p contain leucodelphinidin as do all other combinations involving P^r.

Data from stem material are incomplete. Simultaneous extraction and hydrol-

ysis in boiling 50 percent HCl yields cyanidin from all genotypes tested and a nearly equal quantity of pelargonidin from several types containing *H*. A trace of pelargonidin may be present in *lhP*^{r} stems, and definite traces of delphinidin appear on chromatograms developed in t-butanol. Thus the pattern of extractives from stems analyzed to date follows generally that of the colorless buds. Since *lhP*^{r} stems are red, the pelargonidin in these may be in the form of an anthocyanin rather than a leucoanthocyanin. Pelargonidin is probably derived in part from anthocyanin in *lHP*^{r} and *LHP*^{r} as well as *lhP*^{r}.

Anthocyanidins from anthocyanins: Aqueous solvents removed considerable leucoanthocyanins from tissues. Alcoholic solvents removed less, but nevertheless traces of anthocyanidin from this source could still be obtained in extracts of white flowers. The presence of an anthocyanidin in total extracts which was not represented among the leucoanthocyanins, however, has been considered an indication of its presence as an anthocyanin, especially where this is also indicated by the color of the flower (in Table 2, compare columns b and c with d). Reliable evidence that an anthocyanidin was derived from an anthocyanin was also gained through chromatography of the glycosides followed by elution, hydrolysis, and rechromatography of the aglycones (Table 2, column e). Direct comparison of anthocyanins extends the information to paler flowers where bands were not sufficiently strong for elution and hydrolysis (see Table 3). The anthocyanidins considered present in each genotype as anthocyanins are summarized in the final column of Table 2.

Malvidin occurs in extracts only when *L* is present in the genotype. Pelargonidin always occurs when *H* is present, but is also found in petals when only *P*^{r} or *P*^{g} are present. The pelargonidin producing action of *P*^{r} (or *P*^{g}) is masked by the presence of *L*, although *L* is not epistatic to *H*.

In the sepals, both *P*^{r} and *P*^{g} govern the production of peonidin derivatives. Cyanidin derivatives occur with *P*^{r} but have not been detected with *P*^{g}. These products are apparently eliminated with the introduction of *L* into the genotype but are not eliminated by *H*.

No single gene is essential to anthocyanin formation, for only the multiple recessive is truly white. Of the alleles at the '*p*' locus, *P*^{r} invokes more intense pigmentation than occurs in corresponding combinations involving *P*^{g}, and combinations involving *P*^{g} produce more pigment than those involving *p*. This effect is reflected in the stem where *P*^{r} brings about distinct red coloration through at least the lower-most internode, *P*^{g} provides a faint rose flush in seedling stages and *p* has no apparent effect.

Anthocyanins: Each anthocyanidin is represented in balsams by two or more derivatives. These have been classified through chromatography in the ethyl acetate solvent without prior hydrolysis and are listed for each genotype in Table 3. The major bands of all combinations involving *P*^{r} have been eluted, hydrolyzed, and rechromatographed as aglycones. The bands labeled pelargonidin 3 and cyanidin 2 have not been successfully eluted in sufficient quantity for analysis, but have been assigned on the basis of fluorescence and color after spraying

TABLE 3

Anthocyanins from genetic strains of Impatiens balsamina

	Pelargonidin			Peonidin		Cyanidin			Malvidin	
Genotype	1	2	3	1	2	1	2	3	1	2
Petals										
lhp	..	..	..	..	..	..	..	..	..	..
Lhp	..	..	..	..	..	..	..	..	+	..
lHp	+	+	..	..	..	..	..	..	..	..
LHp	..	..	..	..	..	..	..	..	+	..
lhP^g	+	+	..	..	..	..	..	..	..	..
LhP^g	..	..	..	..	..	..	..	..	+	+
lHP^g	+	+	+	..	..	..	..	..	..	..
LHP^g	+	+	..	..	..	..	..	..	+	+
lhP^r	+	+	..	..	..	..	..	..	..	..
LhP^r	..	..	..	..	..	..	..	..	+	+
lHP^r	+	+	+	..	..	..	..	..	..	..
LHP^r	+	+	+	..	..	..	..	..	+	+
Sepals										
lhp	..	..	..	..	..	..	..	..	..	..
Lhp	..	..	..	..	..	..	..	..	..	..
lHp	..	..	..	..	..	..	..	..	..	..
LHp	..	..	..	..	..	..	..	..	..	..
lhP^g	..	..	..	+	+	..	..	..	..	..
LhP^g	..	..	..	..	..	..	..	..	+	..
lHP^g	+	+	..	..	..	..	..	..	..	..
LHP^g	..	..	..	..	..	..	..	..	+	..
lhP^r	..	..	..	+	+	+	+	+	..	..
LhP^r	..	..	..	..	..	..	..	..	+	+
lHP^r	+	+	..	+	..	+	..	..	..	..
LHP^r	..	..	..	..	..	..	..	..	+	+

with $AlCl_3$. Neither of these is likely to be confused with derivatives of other anthocyanidins known to occur in the same flower parts. In addition, the band labeled pelargonidin one appears to be doubled in some chromatograms of extracts of petals of *lhP*^{r}, *lhP*^{g}, and *lHP*^{r}. This suggests the presence of another pelargonidin derivative.

The anthocyanins present in plants carrying *P*^{g} or *p* have been matched with the more prominent components of the corresponding classes involving *P*^{r}. Where bands are absent in chromatograms of extracts of the more weakly pigmented flowers, the missing bands are always the minor components of extracts of intensely pigmented forms. The absence of such bands suggests a failure to achieve sufficiently concentrated extracts rather than absence of the specific derivatives in the flowers. It is noteworthy, however, that pelargonidin is present in lower concentrations relative to malvidin in the combinations *LHp* and *LHP*^{g} than in *LHP*^{r}. In *LHp*, pelargonidin derivatives were not detected among the anthocyanins although the color of the petals suggests their presence.

DISCUSSION

Genetic regulation of flower color in *Impatiens balsamina* influences the quantity and nature of the anthocyanidin nucleus rather than the nature of the glycosidic or acyl groups attached or such factors as copigmentation and pH of the cell sap. The genes must, therefore, affect specific stages in the synthesis of the anthocyanidins and perhaps of related flavonoids.

The classical hypothesis concerning the origin of these compounds was developed by ROBINSON (1935). A polyphenol resembling phloroglucinol is assumed to combine with a polyhydroxyphenylpropane derivative to yield a precursor resembling I. An alternative proposal by BIRCH and DONOVAN (1953) involves head to tail condensation of three acetate units with an appropriate C_6-C_3 unit. In this way the fused phenyl (A) ring formation would not involve free phloroglucinol.

Strong support for the "acetate theory" has come from recent work. WATKINS *et al.* (1957) have evidence that neither mesoinositol or phloroglucinol is incorporated into rutin in buckwheat, and WEYGAND *et al.* (1957) found that C^{14}-labeled mesoinositol was not incorporated into rubrobrassicin in red cabbage seedlings. Watkins also reported results from the feeding of sodium acetate-1-C^{14} and sodium acetate-2-C^{14} which indicate that labeled acetate is selectively incorporated into the A ring. Finally, GRISEBACH (1957) who likewise used sodium acetate-1-C^{14} and sodium acetate-2-C^{14} found that, in red cabbage seedlings the pattern of C^{14} present in the A ring of rubrobrassicin represents the pattern which would be expected on the basis of the "acetate theory." This pathway could also yield a compound such as I in Figure 1. A cyclic form of this compound would bear a close resemblance to melacacidin (II), a leucoanthocyanin isolated from wood of *Acacia melanoxylon* by KING and BOTTOMLEY (1953). Melacacidin yields an anthocyanidin which is not known in nature, but other leucoanthocyanins are thought to be similar in structure (BATE-SMITH 1953; SWAIN 1954).

The leucoanthocyanins of the balsam have never been characterized. By analogy they are perhaps flavandiols, like melacacidin, with the hydroxylation patterns of pelargonidin, cyanidin, and delphinidin. Through appropriate dehydration and oxidation or reduction the various flavonoid compounds, including flavonols like quercetin (III) and anthocyanidin (IV) could arise. The leucoanthocyanins, however, are not necessarily *in vivo* precursors of the anthocyanins and may represent a class of compounds independently derived from a common precursor resembling I. The five anthocyanidins derived from balsam tissues differ only in the groups appended to the free phenyl ring (IV,B).

In the balsams the series *p*, P^g, and P^r leads to increased anthocyanin production in the order listed when alone or in corresponding combinations with *L* and *H*. Since several anthocyanidins occur in plants carrying P^r or P^g as the only dominant gene and since it is unlikely that the locus would affect both the total anthocyanin production and the specific hydroxylation or methoxylation pattern, the gene action must allow anthocyanin production from fragments of appropriate structure which are present at the time of synthesis. The distinction be-

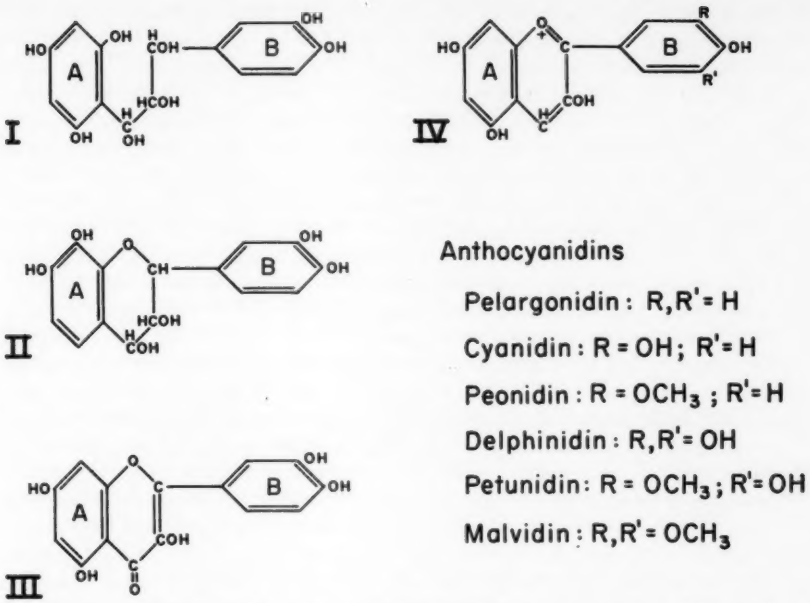

FIGURE 1.—Compounds of interest in the consideration of the biosynthesis of flavonoids: I Hypothetical precursor of the flavonoid nucleus; II melacacidin; III quercetin; IV anthocyanidin.

tween various alleles grouped as P^g or as P^r by DAVIS (1958) lies largely in the extent and pattern of anthocyanin production, so it is probable that even qualitative differences among alleles of *p* reflect differences in the time or extent of gene action rather than specific regulation of ring substitution.

Several possibilities as to the nature of the specific chemical effect of the dominant alleles of *p* are suggested, but a choice between these awaits more data on quantitative relations between the anthocyanins and other flavonoid compounds. Another complicating factor, frequently overlooked, is that theoretical competition between groups of compounds for a common moiety may not actually occur due to differences in time of synthesis of the different types. For example, in the balsam leucoanthocyanins appear in buds much earlier than anthocyanins and somewhat earlier than flavonols.

The genes *H* and P^r must have both an additive and synergistic action. The total pelargonidin production in petals of plants carrying *H* and P^r is considerably greater than the sum of the production in lHp and lhP^r, yet those pigments of sepals which are characteristic of lhP^r persist in lHP^r. Addition of *L* to the complement eliminates the products of P^r but does not eliminate the enhanced pelargonidin production associated with the synergism of *H* and P^r. *H*

must, therefore, make available for anthocyanin synthesis a precursor which is susceptible to the reactions promoted by P^r but which is not formed at the expense of the precursors available to P^r in the absence of H.

At present the stage in the biosynthesis of flavonoids at which the hydroxylation or methoxylation pattern is established is unknown. Moreover, it is uncertain whether the leucoanthocyanins are formed independently from a common precursor or are able to serve directly as precursors for the anthocyanins. In balsams H, but not P^r, favors production of the pelargonidin configuration in both leucoanthocyanins and anthocyanins, but this could represent modification of a common precursor before completion of the flavonoid nucleus. Similar correlation between anthocyanin and leucoanthocyanin structure has been reported previously by COE (1955) in maize and BEALE *et al.* (1939) in sweat pea. If leucoanthocyanins served regularly as precursors for anthocyanins this relation would be expected to hold generally, yet leucoanthocyanins are commonly absent in herbaceous plants (BATE-SMITH 1954) and in the balsams the presence of a leucodelphinidin in buds is not necessarily associated with the production of the corresponding anthocyanidins (delphinidin, malvidin, or petunidin). Moreover, the production of anthocyanins might be accompanied by a decrease in the corresponding leucoanthocyanins as has been reported in bananas (SIMMONDS 1954) and Eucalyptus (HILLIS 1956). The apparent absence of leucoanthocyanin derivatives in aqueous one percent HCl extracts of intensely colored flowers (Table 2) caused the authors to support the concept that leucoanthocyanins were in this case the immediate precursors of anthocyanins (ALSTON and HAGEN 1955). Aqueous solvents, however, remove only a portion of the leucoanthocyanins from balsam tissues and these are obscured on chromatograms by the much greater quantities of anthocyanidins derived from anthocyanins. The application of methods which convert the leucoanthocyanins to anthocyanidins simultaneously with extraction from the tissues, especially after prior removal of the anthocyanins, has revealed the presence of leucoanthocyanins in mature, heavily pigmented flower parts. Attempts to assay the quantities of leucoanthocyanins present at various stages of developing buds have yielded erratic results.

L also affects the substitution of the B ring. Its action differs from that of H, however, in two ways: (1) L does not affect the nature, or, conspicuously, the relative quantities of leucoanthocyanins present, and (2) L through competition or modification eliminates from flowers the pigments produced by P^r (or P^s) in the absence of L. The interpretation is complicated by absence of direct information as to whether the methyl ethers are formed by reactions equivalent to methoxylation or to methylation. If L provides a methoxylated precursor, this must be unavailable for leucoanthocyanin synthesis either through late appearance in the buds or through specific exclusion from the reactions involved. Methoxyl leucoanthocyanins are uncommon in nature although BATE-SMITH (1954) has reported a leucopigment which yields an anthocyanidin resembling peonidin.

The genetic system in *Impatiens balsamina* resembles closely that in *Primula*

sinensis as described by SCOTT-MONCRIEFF (1936). In that species, *v* induces pale flowers and green stems, pure white flowers being obtained only when other genes regulating anthocyanin structure are recessive. In the absence of other dominant genes, *V* allows red stems and pelargonidin derivatives in the flowers. *Dz* produces slight pelargonidin pigmentation with *v* and much more with *V*. *K* replaces the pelargonidin produced by *V* with malvidin, but when both *K* and *Dz* are present pelargonidin and malvidin occur together. Thus *V* of *Primula sinensis* is the equivalent of P^r of the balsams, *K* matches *L*, and D_z has the action of *H*. Other genes of *Primula sinensis* may find their counterparts in *Impatiens balsamina* with *w* which controls a cream background color (but not a copigmentation) and *F* which introduces white spotting of colored petals.

Primula sinensis has recently been re-examined by DAYTON, HARBOURNE, and SHERRAT (1956) who find petunidin and delphinidin as well as malvidin in plants carrying *K*. This does not alter the resemblance to *Impatiens balsamina* but suggests that *K* of Primula and, perhaps, *L* of Impatiens act to introduce the hydroxyl subsituent in the free phenyl ring, the methyl ether formation being independent of the action of these genes.

The effect of the anthocyanin genes on other flavonoids is not yet clear. Identification of the flavonol complement is complicated by a considerable excess of one component, probably kaempferol, which obscures others present in the extracts.

In addition to flavonols, other flavonoid compounds occur and these exhibit changes in relative quantity during bud development.

SUMMARY

Flowers of *Impatiens balsamina* exhibit a wide range of colors controlled by genes at three loci. Each color class yields two or more anthocyanins which may be converted to anthocyanidins by boiling with strong acids. The aglycones can be identified readily through paper chromatography. Both aqueous and alcoholic extracts, however, contain leuco-anthocyanins which yield anthocyanidins under the same conditions which hydrolyze the anthocyanins.

The leucoanthocyanins of balsams have been freed of anthocyanins by extraction of fresh flower parts with alcoholic solvents. The colorless residual tissue releases anthocyanidins derived from leucoanthocyanins when boiled in strongly acid solution. All genotypes yield cyanidin and delphinidin under these conditions from petals and sepals and from immature, colorless buds as well as from mature pigmented flowers. In addition a leucoanthocyanin which yields pelargonidin is present whenever the genotype includes the gene *H*.

Since extracts containing anthocyanins generally include leucoanthocyanins, anthocyanidins obtained by boiling the extracts with acid are of mixed origin. Where the anthocyanidins differ from the derivatives of leucoanthocyanins known to be present, they must be derived from anthocyanins, but where leucoanthocyanins and visible pigments yield the same aglycone, the anthocyanins must be separated by chromatography, eluted, hydrolyzed, and the derived

aglycones identified by chromatography. For technical reasons the latter method has been successful only with the major components of intensely pigmented flowers, although the identifications may be extended to weakly pigmented flowers by directly matching on chromatograms the anthocyanins of the several color classes.

In this manner, ten anthocyanins derived from four anthocyanidins have been identified in the twelve phenotypes available. Only the multiple recessive lacks visible pigment. The genes *H* and *L* lead to the production in petals of small quantities of pelargonidin and malvidin derivatives respectively. P^r as the only dominant pigmentation gene introduces pelargonidin into the petals and cyanidin and peonidin into the sepals. P^s, an allele of P^r, is similar in its action although colors produced are less intense and cyanidin does not appear as a visible pigment.

When *H* and P^r occur together, production of pelargonidin derivatives is much increased, although cyanidin and peonidin in addition to pelargonidin occur in the sepals. *L* is completely epistatic to P^r, malvidin derivatives being produced to the exclusion of pelargonidin, cyanidin, and peonidin. *L* is not epistatic to *H* and when both dominant genes are present flowers yield pelargonidin and malvidin from the petals. Sepals contain only malvidin, however. The presence of P^r, P^s, or *p* affects only the color intensity in all combinations involving *L*. The pattern of pigment inheritance in *Impatiens balsamina* is quite similar to that in *Primula sinensis*.

Genes at the *p* locus apparently regulate a general reaction in anthocyanin synthesis whereas *H* and *L* control specific substitutions in the ring structure. The control of both leucoanthocyanin and anthocyanin structure by the single gene, *H*, implies a close association of the syntheses of the two classes of compounds. While the nature of the relationship is limited to relatively few possibilities, these cannot be resolved by qualitative studies. On the other hand quantitative analyses of flavonoid components during bud development should permit ultimate definition of the biochemical effects of the genes at the three loci.

LITERATURE CITED

ALSTON, R. E., and C. W. HAGEN, JR., 1955 Relation of leucoanthocyanins to anthocyanin synthesis. Nature **175**: 990.

BATE-SMITH, E. C., 1948 Paper chromatography of anthocyanins and related substances in petal extracts. Nature **161**: 835.

1949 Anthocyanins, flavones and other phenolic compounds. Biochem. Soc. London. Symp. **3**: 62–73.

1953 Colour reactions of flowers attributed to (a) flavanols and (b) carotenoid oxides. J. Exp. Bot. **4**: 1–9.

1954 Leucoanthocyanins 2. Systematic distribution of leucoanthocyanins in leaves. Biochem. J. **58**: 126–132.

BEALE, G. H., J. R. PRICE, and V. C. STURGESS, 1941 A survey of the anthrocyanins. VII. The natural selection of flower colour. Proc. Roy. Soc. London B **130**: 113–126.

BEALE, G. H., G. M. ROBINSON, R. ROBINSON, and R. SCOTT-MONCRIEFF, 1939 Genetics and chemistry of flower colour variation in *Lathyrus odoratus*. J. Genet. **37**: 375–388.

BIRCH, A. J., and F. W. DONOVAN, 1953 Studies in relation to biosynthesis. I. Some possible routes to derivatives of orcinol and phloroglucinol. Aust. J. Chem. **6**: 360–368.

COE, E. H., 1955 Anthocyanin synthesis in maize, the interaction of A_2 and p^r in leucoanthocyanin accumulation. Genetics Soc. of America, Records No. **24**: 568.

DAVIS, D. W., LUCY A. TAYLOR, and ROY P. ASH, 1958 *Impatiens balsamina* L.—The inheritance of floral colors. Genetics **43**: 16–34.

DAYTON, T. O., J. B. HARBOURNE, and H. S. A. SHERRAT, 1956 Biochemical genetics of the flower pigments of *Primula sinensis*. Biochem. J. **63**: 29.

FORSYTH, W. G. C., and N. W. SIMMONDS, 1954 A survey of the anthocyanins of some tropical plants. Proc. Roy. Soc. London B **142**: 549–564.

GRISEBACH, HANS, 1957 Zur Biogenese des Cyanidins I. Mitt.: Versuche mit Acetat-[1-^{14}C] und Acetat-[2-^{14}C]. Z. Naturforschung **12(4)**: 227–231.

HAYASHI, K., Y. ABE, T. NOGUCHI, and K. SUZISHINO, 1953 Studien über Anthocyane. XXII. Beiträge zur papierchromatographischen Analyse der natürlichen Anthocyane und Anwendung derselben zur Untersuchung von Farbstoffen in den roten *Impatiens*—Blüten und den blutroten Pfirsich—Früchten. Pharm. Bull. **1**: 130–134.

HILLIS, W. E., 1956 Leucoanthocyanins as the possible precursors of extractives in woody tissues. Australian J. Biol. Sci. **9**: 263–280.

KING, F. E., and W. BOTTOMLEY, 1953 The isolation from *Acacia melanoxylon* of a flavan—3:4—diol and its possible bearing on the constitution of phlobatannins. Chem. and Ind. **1953**: 1368.

ROBINSON, G. M., and R. ROBINSON, 1931 A survey of the anthocyanins I. Biochem. J. **25**: 1687–1705.

ROBINSON, R., 1935 Formation of anthocyanins in plants. Nature **135**: 732.

SCOTT-MONCRIEFF, ROSE, 1931 The chemical effects of a Mendelian factor for flower colour. Nature **127**: 974–975.

1936 A biochemical survey of some Mendelian factors for flower colour. J. Genet. **32**: 117–170.

SIMMONDS, N. W., 1954 Anthocyanins in bananas. Nature **173**: 402–403.

SWAIN, T., 1954 Leucocyanidin. Chem. and Ind. **1954**: 1144–1145.

WATKINS, J. E., E. W. UNDERHILL, and A. C. NEISH, 1957 Biosynthesis of quercetin in buckwheat. Part II. Can. J. Biochem. Physiol. **35(3)**: 229–237.

WEYGAND, FRIEDRICH, WOLFGANG BRUCKER, HANS GRISEBACH and EBERHARD SCHULZE, 1957 Stoffwechselnunterschungen mit meso-Inosit-^{14}C. Z. Naturforschung **12(4)**: 222–226.

WILLIAMS, R. J., and H. KIRBY, 1948 Paper chromatography using capillary ascent. Science **107**: 481–483.

A COMPARISON OF CROSSOVER RELATIONSHIPS IN A DROSOPHILA RING X CHROMOSOME AND A ROD X DERIVATIVE[1]

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TWO stable ring X chromosomes are known in *Drosophila melanogaster*. The first, which was given the symbol X^{c1}, y, was discovered by MORGAN in 1922 (MORGAN 1926, 1933). This chromosome was recovered in a female from among the progeny of an attached-X female; the yellow body-color mutation is inseparable from the ring. The second ring X is called X^{c2}, and was discovered by R. D. BOCHE (BEADLE and STURTEVANT 1935). It was also recovered from an attached-X stock. X^{c2} carries no inseparable markers. SCHULTZ and CATCHESIDE (1937) have analyzed both ring chromosomes, using salivary gland chromosome smears. X^{c1}, y was found to be deficient for the tip from band 1A8 on. The broken end is joined to the base of the chromosome in the region between 20A and 20C. The X^{c2} chromosome is also deficient for the tip and both chromosomes have duplications for a part of region 20; X^{c1}, y for 20C and D approximately. Presumably both ring X chromosomes arose as a result of a break in one arm of the attached-X near the tip and in the other arm near the base, with subsequent union of the broken ends in such a way as to lead to the formation of a ring and an acentric rod. This manner of origin explains the presence of the proximal duplication in each chromosome.

The ring chromosomes offer an opportunity for the study of some of the problems of crossing over. Heterozygotes between either ring X and any rod X chromosome yield only double or other even-numbered crossovers among their offspring (MORGAN 1933; STURTEVANT and BEADLE 1936). It was at first believed that egg mortality was responsible for this effect, because with an odd number of exchanges the chromatids must be either acentric or dicentric. STURTEVANT and BEADLE showed, however, that such odd-numbered exchange chromatids are never included in the fertilization nucleus of the mature egg. The reason for this lies in the alignment of the maturation spindles in the egg of Drosophila, as first described by HUETTNER (1924). Of the four nuclei formed by the maturation divisions, the innermost one always becomes the functional female pronucleus, and, for mechanical reasons, receives a normal chromatid, if any. Thus, only chromatids with an even number of exchanges can be recovered.

The rate of double crossing over in ring/rod heterozygotes has been measured for X^{c1}, y by MORGAN (1933) and for X^{c2} by NOVITSKI (1952). MORGAN's infor-

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mation on X^{c1}, y which is quite extensive, includes data on both X^{c1}, y/rod X heterozygotes and X^{c1}, y homozygotes. In both types the double crossover values were less than one half of the expected values. This depression in the frequency of double crossovers is most marked at the "ends" of the chromosome, a finding which is not unexpected, as proximity to the proximal heterochromatin and to the ends of a rearrangement are known to decrease crossing over. NOVITSKI's data on double crossing over in X^{c2} heterozygotes show substantially the same result. For the central region of the chromosome his values are about 100 percent of the expected, but for the two "ends" the observed values are only 5 percent and 31 percent of the respective expected values.

The observed depression in double crossover values might be connected with the proximal heterochromatic duplication, or, on the other hand, it might result entirely from the ring structure itself. A method for investigating these possibilities presented itself when it was observed that the chromosome *In(1)* X^{c1}, y, which arose spontaneously from X^{c1}, y, likewise gave depressed double crossover values when heterozygous with a normal-order rod. This fact alone eliminated the possibility that the ring structure itself was responsible, and the fact that crossing over could be measured in *In(1)* X^{c1}, y heterozygotes made it possible to test the alternative possibility.

RESULTS

The In(1) X^{c1}, y *chromosome*

The chromosome *In(1)* X^{c1},y was discovered in a stock of X^{c1}, y. In genetic tests the new chromosome acted like an inverted rod. In the progeny of crosses involving heterozygotes between this chromosome and rods with normal gene order, only double crossover occurred (table 1) but the supposed rings which carried double crossovers in the F_1 were almost as frequent as the rods with double crossovers. This is not the case when a ring/rod heterozygote is tested. Salivary gland smears showed an apparently normal X^{c1}, y chromosome, with both the tip deficiency and the proximal duplication. In larval neuroblast smears, however,

TABLE 1

F_1 of the cross In(1) *X^{c1}*, y/sc cv v f × sc cv v f sp-f
*Region: (1) (2)(3)(4)**

Region	None	Single	Crossovers: Double 1-2	1-3	1-4	2-3	2-4	3-4	Total
No. obs.	2,658	0	19	44	21	38	48	12	2,840
†Corr. no.	....	0	31	74	35	64	80	20	2,901
Percent	....	0.0	1.1	2.5	1.2	2.2	2.8	0.7	....
†Percent exp.	....	0.0	1.9	3.3	1.3	3.2	1.8	1.5	....
Ratio $\frac{\text{obs.}}{\text{exp.}}$	....	.	0.6	0.8	0.9	0.7	1.6	0.5	....

* See text for explanation of the assumed crossovers involving region 4.
† See text for method of correction of observed number and for method of calculation of expected percentages.

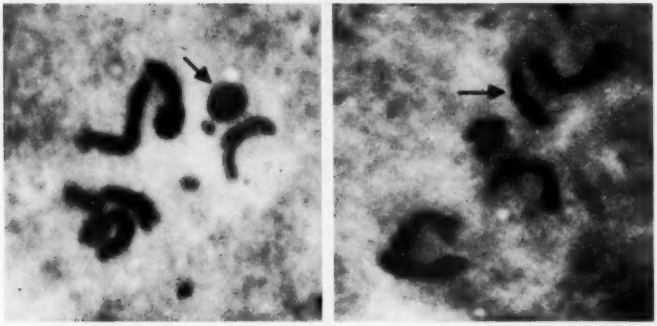

FIGURE 1.—Neuroblast mitotic metaphase of an X^{c1}, y male larva (left) and an *In(1)* X^{c1}, y male larva (right). Arrows indicate X chromosomes. Aceto-orcein smears, 3600×.

the chromosome was rod-shaped (Fig. 1). It was concluded that the ring had probably broken at its former base and thus, since it would now have its centromere at the y end, constituted a "total inversion", at least for the euchromatic length. In the larval neuroblast prophase the new chromosome has a fair-sized heterochromatic portion at the tip of its left arm. The right arm is very small, as is the case with a normal X chromosome. A diagram of *In(1)* X^{c1}, y appears in Figure 2. It seems likely that the heterochromatic tip is the duplicated portion of region 20 of the parent X^{c1}, y chromosome. As no tests have been made, however, one cannot exclude the possibility that the event which led to the opening of X^{c1}, y was an X-Y or an X-4 translocation, and that the distal heterochromatic portion of *In(1)* X^{c1}, y is Y- or 4-heterochromatin. NOVITSKI (1949) has found a similar opened X^{c1}, y chromosome, called *In(1)EN*. That *In(1)EN* is probably not identical with *In(1)* X^{c1}, y is shown by the fact that *In(1)EN* appears in the neuroblast metaphase as a rod X "plus a short arm about one fourth the length of the longer arm of the X."

Crossing over between In(1) X^{c1}, y *and a normal rod X*

Females which were heterozygous for *In(1)* X^{c1}, y and a normal order rod X bearing the markers *sc*, *cv*, *v*, and *f* were backcrossed to *sc cv v f* males in mass cultures. The P_1 females were allowed to lay eggs for five days, and then discarded. The F_1, counted for nine days from the first day of hatching, was virtually complete, since few if any flies eclosed on the last two days. The results of the experiment are shown in Table 1. The results for the two sexes were not different and are pooled. Since an inversion heterozygote can produce only double or higher order even-numbered exchange chromatids, all apparent singles have been scored as doubles with one of the crossovers in the unmarked region between *f* and *sp-f* (region 4). As there were no crossovers between y and *sc*, this region was not numbered in the table. The observed number of double crossovers is not, of course, a direct measure of exchange frequencies. Some three-strand and all four-strand double crossovers lead to the loss of the X chromosome from the egg. If the

nullo-X egg is fertilized by an X-bearing sperm, a patroclinous male is produced, while fertilization by a Y-bearing sperm produces a lethal Y/O zygote. Patroclinous males have been shown to occur in a ratio of two for every three double exchange males, and presumably Y/O zygotes occur in a ratio of 2:3 of the double exchange females. In the matings shown in Table 1, patroclinous males cannot be detected. The observed numbers are, therefore, corrected for the expected patroclinous males and lethal Y/O zygotes. The exchange pe centages are calculated from these corrected exchange offspring numbers. The expected exchange figures in the table were calculated by multiplying the standard map distances (BRIDGES and BREHME 1944) for the regions involved, and then correcting for interference by assuming an arbitrary coincidence value of 0.7 for adjacent regions. No interference was assumed for nonadjacent regions. The data of Table 1 show that when the above-mentioned factors are taken into account, the double crossover values are lower than expected for adjacent regions and higher than expected for double exchanges involving regions 2 and 4.

Crossing over between In(1) X^{c1}, y *and other inversions*

While only even-numbered multiple exchanges are to be expected in inversion heterozygotes, inversion homozygotes yield both odd and even-numbered exchanges. Because of this fact, it is possible to obtain single crossover values for *In(1)* X^{c1}, *y*. One peculiarity of single crossovers in heterozygotes for *different* inversions, however, is that single or higher order odd-numbered exchanges produce one duplicated and one deficient chromatid. The duplications and deficiencies are for those portions of the length of the chromosome included in one inversion but not in the other. Where the proximal breaks coincide, or nearly coincide, in the heterochromatin, one exchange chromatid is viable in the male, while the other one is lethal. In the case of a single crossover within the common inverted segment between *In(1)* y^{3P} and *In(1)* w^{m4}, for example, one of the exchange chromatids would have a duplication for the region between 2C1-2 (the left break point of *In(1)* y^{3P}) and 3C2-3 (the left break point of *In(1)* w^{m4}) of the salivary gland chromosome. In one crossover chromosome this region would appear once in its normal position just distal to the inversion, and once at the proximal end of the inversion. In this example there would also be a slight duplication for some of the proximal heterochromatin, since the right break points of the two inversions do not exactly coincide. The reciprocal exchange chromatid would, of course, be deficient for the same regions.

In such cases the crossover frequency may be measured by dividing the number in the duplication class of the exchanges in a particular region by the sum of the duplication class *(A)* plus one half of the parental classes *(B)*, as follows:

$$\text{Crossover frequency} = \frac{A}{A + \frac{B}{2}}.$$

In crosses where there are large viability differences between the parental chromo-

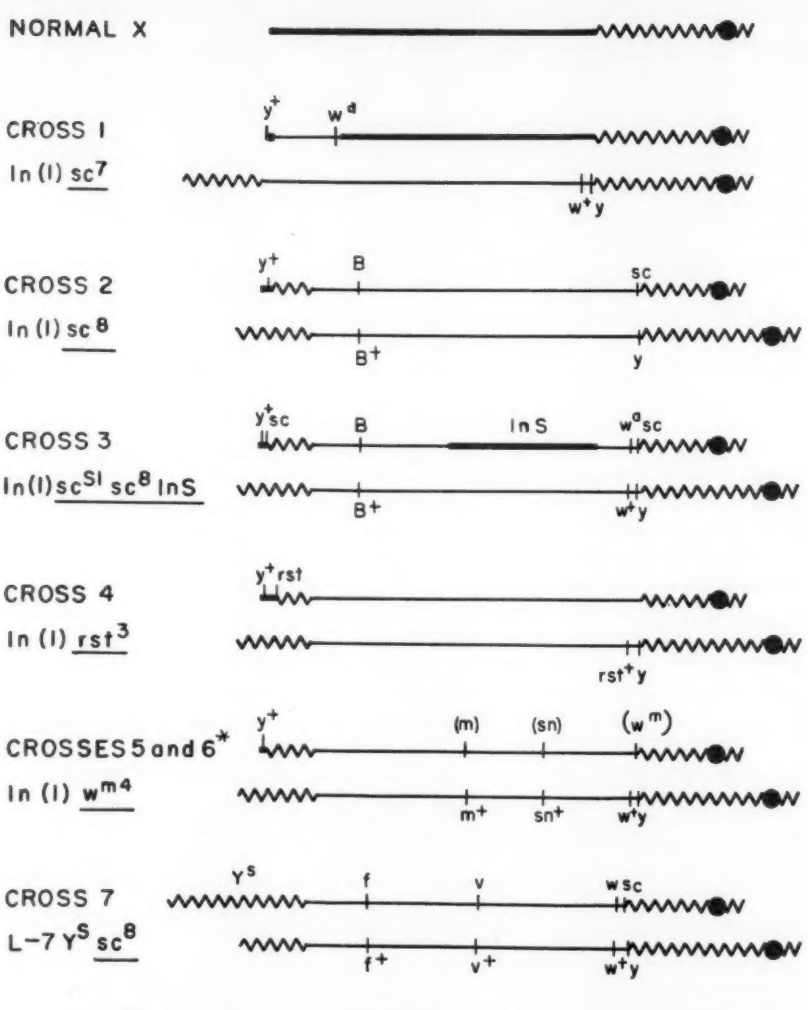

FIGURE 2.—Schematic maps of the X chromosome inversions used in crosses 1 through 7, Table 2. The lower chromosome in each case is *In(1)* X^{ct}, *y*.

somes, a better figure is obtained by substituting for $\frac{B}{2}$ the total in that parental class which matches *A* most closely in genotype.

In order to test the single crossover frequency in *In(1)* X^{c1}, *y*, seven different crosses were made, using seven different inversions of the X chromosome (See Fig. 2). In all of these experiments females that were heterozygous for *In(1)* X^{c1}, *y* and the tested inversion were mated to Muller-5 males. The Muller-5 chromosome is composed of the right end of *In(1) sc*^{8} and the left end of *In(1) sc*^{S1}, including the small inner inversion *InS* (SPENCER and STERN 1948). It is marked with *B* and *w*^{a}. The results of all seven experiments are presented in Table 2.

In cross number one, *In(1) sc*^{7} was used. This inversion has its left break just to the right of *sc* and its right break between *cv* and *ct* (DUBININ 1933).

In cross number two, *In(1) sc*^{8} was used. The left break of this inversion is just to the left of *sc* and the right break is in the proximal heterochromatin, between Block A and the centromere (SIDOROV 1936). The wild type male class is composed of two types; *y*, *y*$^{+}$ single crossovers between *B* and the original right breakpoint of *In(1) sc*^{8}; and double crossovers with one exchange between *y* and *B* and the other exchange between *B* and the right breakpoint of *In(1) sc*^{8}. Since *In(1)* X^{c1} shows a slight scute effect, it was not possible to distinguish between the two types. The *y B* F_1 male class is the reciprocal of the double crossover class just described. The single *w*^{a} *B* male was sterile, and is thus taken to be patroclinous.

The third cross makes use of the Muller-5 chromosome, already described above. The left break of the larger *sc*^{S1} inversion is just to the right of *sc*, and the right break is at about the same level as that of *sc*^{8}, in the proximal heterochromatin. The inner inversion, *InS*, has its left break to the right of *rb* and its right break to the left of *g* (CREW and LAMY 1940). Among the F_1 males, the + class represents single crossovers between the original right breakpoint of *In(1) sc*^{8} and *B*. The *B* male class represents single crossovers between *w*^{a} and *B*. The reciprocals of both single crossover classes are lethal, because they are deficient for the tip up to *sc*. The *w*^{a} males and the *y B* males probably are the reciprocal double crossovers with one exchange in each of the regions described above. The $\frac{B}{+}$ *w*^{a} and $\frac{B}{B}$ female classes also represent the two reciprocal double exchange classes. The *y* $\frac{B}{+}$ female was probably the result of a *y* mutation in the Muller-5 chromosome, or of equational nondisjunction with a rare double crossover in one chromatid.

In cross number four, *In(1) rst*^{3} was used. This inversion has its left break at *rst;* the right break is in the proximal heterochromatin (GRUNEBERG 1937). The + male F_1 class here represents single crossovers within the *rst* inversion, with the left end of the *In(1) rst*^{3} chromosome and the right end of the *In(1)* X^{c1}, *y* chromosome. They carry a duplication of the segment tip-*rst*. The five *B w*^{a} males were patroclinous. The other exceptional classes may all be explained by mutation or equational nondisjunction.

In both crosses five and six, use was made of *In(1) w^{m4}*. This inversion has its left break to the left of *w* and its right break between *bb* and Block A in the proximal heterochromatin (MULLER 1930). In the fifth cross, single exchanges within the inverted region of *In(1) w^{m4}* produce the + class of duplication males. The complementary crossover males are absent, as would be expected, since they would be deficient for the tip up to the *w* locus. The w^a *B* male was patroclinous.

TABLE 2

F_1 of seven crosses described in the text

Cross

1. *In(1) sc^7, w^a/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{+}$ w^a ♀	$\frac{B}{+}$ ♀	*y* ♂	w^a ♂	+ ♂
No.	524	519	441	290	0
%	29.5	29.2	24.8	16.4	0.0

2. *In(1) sc^8, B/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{B}$ ♀	$\frac{B}{+}$ ♀	*y* ♂	*B* ♂	+ ♂	*y B* ♂	w^a *B* ♂
No.	1578	1924	930	1216	226	69	1
%	25.6	32.5	15.7	20.5	4.5	1.2	

3. *Ins(1) sc^8 sc^{81} InS, B w^a/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{B}$ w^a ♀	$\frac{B}{+}$ ♀	*y* ♂	w^a *B* ♂	+ ♂	$\frac{B}{B}$ ♀	$\frac{B}{+}$ w^a ♀	*B* ♂	w^a ♂	*y B* ♂	$\frac{B}{+}$ *y* ♀
No.	7345	10105	8170	6107	1137	339	372	302	25	2	1
%	21.6	29.8	24.1	17.9	3.3	1.2	1.1	1.0			

4. *In(1) rst^3/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{+}$ ♀	*y* ♂	*rst* ♂	+ ♂	w^a *B* ♂	+ ♀	*rst* ♀	*y* ♀	*y rst* ♂
No.	5701	1963	2150	1243	5	104	59	1	1
%	50.8	17.5	19.2	11.1					

5. *In(1) w^{m4}/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{+}$ ♀	*y* ♂	w^m ♂	+ ♂	w^a *B* ♂	*y* w^m ♀
No.	4082	1489	1547	855	1	1
%	51.1	18.6	19.3	10.7		

6. *In(1) w^{m4}, y w sn m/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{+}$ ♀	$\frac{B\ w^a}{+\ w}$ ♀	*y* ♂	*y w sn m* ♂	*y sn m* ♂	*y m* ♂	*y w* ♂	*y w sn* ♂	*y* ♀
No.	483	305	309	315	19	20	9	2	87
%	31.2	19.7	19.9	20.3					

7. L-7 Y^s *f v w sc^8/In(1)* X^{c1}, *y* ♀ × Muller-5 ♂

F_1	$\frac{B}{+}$ ♀	*y* ♂	*y v f* ♂	*y f* ♂	*y v* ♂	*f* ♂
No.	5424	1900	458	374	94	1
%	65.7	23.0	5.6	4.5	1.1	

The $y\ w^m$ female was probably the result of an equational nondisjunction with a rare crossover between y and the left inversion point of *In(1) w^{m4}*.

In the sixth cross the white-mottled character could not be scored because of the presence of *w*. In this cross there was no + class, since *y* is present in both chromosomes. Here the F_1 males with the phenotypes yellow, singed, miniature and yellow, miniature are taken to be duplication carrying single crossovers between *w* and *sn*, and between *sn* and *m*, respectively. Genetic tests made of 12 of the yellow females showed them to be products of nondisjunction. The *y w* and *y w sn* males must be double crossover classes for the regions *w-sn* and *m* to the distal end of the inverted segment of *In(1) w^{m4}*, and for the regions *sn-m* and *m* to the end of the inversion, respectively.

For the seventh cross, the chromosome L-7 Y^s *f v w sc^8* was kindly provided by Dr. D. L. Lindsley (Dr. Lindsley also provided the chromosome Y^s *y w^a cv v f* · Y^L, which will be described below). This chromosome is a compound of the *In(1) sc^3* chromosome, marked with *f*, *v*, and *w*, with the L-7 Y^s fragment substituted for the uninverted sc^8 tip. L-7 Y^s is a Y chromosome short arm described by Lindsley (1955). In this cross no F_1 sc^8 male types are to be expected, since the L-7 Y^s *f v w sc^8* chromosome is lethal in the male unless covered by sc^8 · Y. The lack of $\frac{B}{+}$ w^a females is due to an unexplained lack of expression of the w^a *w* phenotype, as shown by the large number of $\frac{B}{+}$ females. The *y v f* F_1 males are single crossovers for the region between *w* and *v*, and the *y f* males are single crossovers for the region between *v* and *f*. The *y v* F_1 males are double crossovers for both of these regions. The *f* male remains unexplained.

In order to make sure that the + F_1 male classes of Crosses 2–5 (Table 2) really carry *y*, y^+ duplication single crossover chromosomes, a backcross was made in which the deficient *y* reciprocal single crossover chromosome could be detected in the female. In a cross *In(1) sc^8, B/In(1)* X^{c1}, *y* females by *y* males, one half of any *y*-deficient chromosomes produced by a crossover to the left of *B* can be detected in *Ins(1)* X^{c1} *sc^8, y⁻ B/y* females. These females will have the phenotype yellow heterozygous Bar. Of 5,228 F_1 females from that cross 452 had such a phenotype. Of these females, 32 were tested by mating them to *y/y^+ sc^8* · Y males; of 24 successful tests, all produced both Bar and wild type males in equal numbers. Hence the reason for the lack of a yellow Bar class among the F_1 males of Cross 2, for instance, must be that the chromosome is deficient for a segment which is "covered" by sc^8 · Y, but not by a normal Y.

Information about the frequency of crossing over between *B* and the distal end of the inversion in *In(1) sc^8, B/In(1)* X^{c1}, *y* females may be extracted from the female F_1 counts of the experiment just described. Of the total 5,228 F_1 females, there were 452 yellow deficiency $\frac{B}{+}$ females and 321 duplication wild type females. The crossover frequency between *B* and the distal end of the inversion thus amounts to 14.8 percent.

Table 3 presents the crossover information which may be derived from the raw data of Table 2 as well as the testcross just considered. The expected values are simply the standard map distances between the markers involved. It will be seen that the observed/expected ratios fall into three general classes: those above 1, those between 0.6 and 0.7, and those below 0.3. The three which are above one are all for measurements between *B* and the left end of the inverted segment of the inversion. The reason for the depression in Cross 3, relative to Cross 2 and the testcross, is no doubt the presence of *InS* within the longer inversion. Two crosses yield ratios of 0.6 for measurements of substantially the whole euchromatic length of the chromosome, namely Crosses 4 and 5. The two ratios which lie below 0.3 are for the right (original left) end or the middle of the chromosome, regions moved nearer to the centromere by the inversions. In Cross 7, substitution of L-7 Y^S for the normally proximal heterochromatin of the left end of the inversion more than doubles the value for the v — w region, which might be expected on the basis of the m — w region of Cross 6, and raises it to the 0.6 to 0.7 level.

Crossing over between In(1) X^{c1}, y *and a normal-order X with a heterochromatic left end*

Since the most obvious difference between Crosses 6 and 7 is that of the amount of heterochromatin at the left end of the inverted homologue of *In(1)* X^{c1}, y, it might be supposed that the difference in crossing over was attributable to an effect of the heterochromatin. To test this possibility extra heterochromatin was added to a normal-order X. For this purpose the chromosome Y^S *y* w^a *cv v f* · Y^L was used. This chromosome has heterochromatin in the form of Y^S and Y^L added to both ends. The result is reported in Table 4. Since a backcross cannot be made easily (because one cannot obtain homozygous Y^S *y* w^a *cv v f* · Y^L females), the data include only the F_1 males. These results do not appear to differ markedly from those obtained using a normal X (Table 1). Unlike the results of

TABLE 3

Crossover data which may be extracted from table 2 and the testcross described in the text

Cross	Chromosome	Length	Obs. percent	Exp. percent	Obs./Exp.
1.	*In(1) sc*^{7}, w^a	end*—w^a	0.0	14.4	...
2.	*In(1) sc*^{8}, *B*	end*—*B*	19.6	9.3	2.1
Testcross	*In(1) sc*^{8}, *B*	end*—*B*	14.8	9.3	1.6
3.	*Ins(1) sc*^{8} *sc*^{81} *InS, B* w^a	end*—*B*	11.3	9.3	1.2
4.	*In(1) rst*^{3}	end*—*rst*	38.8	64.3	0.6
5.	*In(1)* w^{m4}	end*—*w*	36.5	64.5	0.6
6.	*In(1)* w^{m4}, *y w sn m*	*m—sn*	3.3	15.1	0.2
		sn—w	4.2	19.5	0.2
7.	L-7 Y^S *f v w sc*^{8}	*f—v*	16.6	23.7	0.7
		v—w	19.5	31.5	0.6

* end = present distal end (original right breakpoint) of inverted segment.

Cross 7, they do not indicate any effect of the extra heterochromatin added to the left end of the normal X chromosome.

Construction and testing of In(1) sc^{sl^L} X^{c1^R}, y y^+

Because of the effect of additional heterochromatin on crosing over observed in Cross 7 (Table 3), and of the apparent lack of any effect of extra heterochromatin on crossing over between *In(1)* X^{c1}, *y* and Y^s *y* w^a *cv v f* · Y^L, a cross was made to test the effect of substituting other hetehochromatin for the distal heterochromatin of *In(1)* X^{c1}, *y*. The necessary chromosome was ready to hand, in the form of one of the + chromosomes derived from Cross 3 (Table 2). It had the left end of *In(1)* sc^{sl}, and the right end of *In(1)* X^{c1}, *y*. A standard crossover test was made for recombination between this chromosome [*In(1)* sc^{sl^L} X^{c1^R}, *y* y^+] and a normal-order rod-X marked with *y* ct^6 ras^2 *f*. Since no significant difference was observed between the frequencies of crossing over as measured in F_1 males and females, they are combined in Table 5. Once again crossover frequencies do not differ obviously from those obtained when *In(1)* X^{c1}, *y* is heterozygous with a normal-order rod X. This result indicates that the peculiar crossover behavior of *In(1)* X^{c1}, *y* cannot be due to some peculiarity of its own distal heterochromatin, which in the present cross has been replaced.

DISCUSSION

As previously pointed out, *In(1)* X^{c1}, *y* yields essentially the same lowered values for double crossing over with an uninverted rod X that the ring chromosomes X^{c1}, *y* and X^{c2} do. In Table 1 the double crossover classes which show the greatest depression below the expected values may be recognized as those which have the two crossovers in adjacent regions. In other words, the interference value is higher than the value allowed in calculating the table. MORGAN (1933), by adding all double crossovers involving a given region, obtained relative measures of the single exchanges for each region. This procedure tends to reduce the effect of interference on values for particular regions. She reported her double crossover data for X^{c1}, *y*/rod X heterozygotes as percentages of exchanges relative to the standard lengths of each region. This figure provides a measure of the depression in exchange frequency. In MORGAN's experiments, this depression was most marked in the middle of the chromosome, and was least marked at the *bb* end. The values for the ratio of the observed percentage of exchanges to the expected (as based on standard map distances and on assumption of no interference) ranged from 0.2 to 0.4. These figures become somewhat lower if one takes into account the fact that double exchange ring-bearing offspring are only about one half as frequent as double exchange rod-bearing offspring. The test of the data from Table 1, if recalculated in the same way, give values of 0.19, 0.14, and 0.30 for regions 2, 3, and 4 respectively. These values agree quite well with MORGAN's data. The data of NOVITSKI (1952) on X^{c2}, when recalculated in this manner, give values of 0.23, 0.25, 0.22, and 0.41, for the regions between *y* and

cv, *cv* and *v*, *v* and *f*, and *f* and *sp-f*, respectively. These figures also agree with the present data fairly well. No allowance has, of course, been made for undetected Y/O lethal zygotes or for patroclinous males. When these classes are allowed for, the above values are raised considerably. Whether or not these corrections are made, however, it is clear that all three chromosomes. X^{c1}, X^{c2} and *In(1)* X^{c1}, *y*, are alike in showing high interference values, and a marked reduction in total exchanges at the distal (*y*) end.

The crossover data obtained from single crossing over between *In(1)* X^{c1}, *y* and other inversions (Table 3) likewise show the highest exchange frequency for the *bb* end of the chromosome. Crossovers between *B* and the original proximal end of the inversion are in fact much more frequent than would be expected from the standard map distance. With *In(1)* sc^s, for instance, the value for the region normally to the left of *B* is only 14.8 percent, or 30 percent of the expected value, while that for the region normally to the right of *B* is 19.6 percent, or 210 percent of the expected value. OFFERMANN, STONE and MULLER (1931) and OFFERMANN and MULLER (1933) in a study of crossing over within the inverted segment in *In(1)* sc^s homozygotes, found that crossing over in the *w* — *ct* region, which was moved much closer to the centromere, was reduced to 65 percent of normal; whereas crossing over in the *m* — *f* region, moved much farther away from the centromere, was above normal. They also found that the *y* — *car* region which in the *In(1)* sc^s chromosome includes a long section of normally proximal heterochromatin but very little normal crossing over distance, still showed very little crossing over. Similar tests of *In(1)* dl-49 homozygotes showed no change in crossover values, either within or on either side of the inverted segment. In similar experiments with homozygous inversions, MATHER (1939) has found a similar increase in crossing over near the *bb* end of the chromosome, whenever it was moved farther away from the centromere. Homozygotes for *In(1)* sc^s gave a value of about 15 percent for the region between *f* and *bb*, or 160 percent of the expected. Homozygotes for *In(1)* rst^3 gave a value of 24 percent for roughly the same region, or an increase to 260 percent of the expected. For no region, however, do MATHER's figures fall appreciably below the expected value.

Since the crossover frequency near the *bb* end of the chromosome in the data given in Table 3 is much greater than expected, it may be assumed that the value of 0.6 for the whole length of the rst^s and w^{m4} inversions is a compound of values greater than expected for the *bb* end and values of 0.2 to 0.3 times the expected, such as were found for originally distal regions in cross 6, for the rest of the inverted segment of the chromosome.

The over-all depression of double crossover frequencies and high interference values characteristic of the ring X chromosomes of Drosophila cannot be caused by the ring shape itself, if the derivative rod chromosome *In(1)* X^{c1}, *y* shows them likewise—and it does. The fact that the *In(1)* sc^{S1} X^{c1}, *y y* + rod chromosome also gives similar crossover frequencies (Table 5) shows that no "factor" of unknown nature, causing this phenomenon is located in the distal end of the *In(1)* X^{c1}, *y*

TABLE 4

F_1 of the cross In(1) X^{c1}, y/Y^S y w^a cv v f · Y^L × *Muller-5*
Region: (1)(2)(3)(4)

Region	None	Single	Crossovers Double 1-2	1-3	1-4	2-3	2-4	3-4	Total males
No. obs.	2,982	0	3	46	36	90	79	8	3,244
†Corr. no.	....	0	5	77	60	150	132	13	3,419
Percent	....	0.0	0.1	2.3	1.8	4.4	3.9	0.4	....
†Percent exp.	....	0.0	1.9	3.3	1.3	3.2	1.8	1.5	....
Ratio obs./exp.	....		0.1	0.7	1.4	1.4	2.2	0.3	....

Data for male F_1 only
Total $F_1 = 7{,}138$

† These figures were calculated as for table 1.

TABLE 5

F_1 of the cross In(1) sc^{S1} X^{c1}, y y+/y ct^6 ras^2 f × y ct^6 ras^2 f sp-f
Region: (1) (2) (3)(4)

Region	None	Single	Crossovers Double 1-2	1-3	1-4	2-3	2-4	3-4	Total
No. obs.	13,468	0	41	458	242	192	190	143	14,734
†Corr. no.	....	0	69	763	403	320	316	238	15,156
Percent	....	0.0	0.5	5.0	2.7	2.1	2.1	1.6	....
†Percent exp.	....	0.0	1.8	4.8	1.9	2.1	1.2	1.5	....
Ratio obs./exp.	....		0.3	1.0	1.4	1.0	1.7	1.1	....

† These figures were calculated as for table 1.

chromosome. Such a "factor," if one exists, must be located at the centromere end of the chromosome.

Several authors have described differences in crossover frequency in the X chromosome which are attributable to the proximal heterochromatin of the X, or to the heterochromatic Y chromosome. STURTEVANT and BEADLE (1936), recalculating data from BRIDGES and OLBRYCHT (1926), found that the addition of a Y chromosome to rod X females had no effect on the crossover frequencies of the *sc* to *v* region, but increased the value for the region from *v* to *g* to 112 percent of the standard value, and that for the region from *g* to *f* to 127 percent of the expected amount. From their own data on inversion heterozygotes, they found that the addition of a Y chromosome has no effect on the single crossover frequencies in *In(1)* Df (*bb*)/+ or *In(1)* y^4/*In (1)* Df (*bb*) heterozygotes. Data on *In(1)* sc^4/+. *In(1)* y^4/+ and *In(1)* dl-49/+ heterozygotes indicated that the presence of a Y chromosome increases crossing over within the inversion and decreases crossing over between the end of the inversion and the centromere.

STERN (1936) reported that the presence of an extra Y chromosome in females increased the rate of somatic crossing over between the X's.

Cross 7 (Table 3) gives us information about the effect of extra Y-heterochromatin on crossing over in *In(1)* X^{c1}, *y*. L-7 Y^{s}, when added to the distal end of the sc^{s} inversion, increased the crossover frequencies in the region between *f* and *w* to more than twice the value for a similar region using *In(1)* sc^{s} alone. The negligible effect of the attached-Y chromosome on double crossing over between *In(1)* X^{c1}, *y* and a normal-order rod is shown in Table 4. The exchange frequencies expressed as percentages of exchange per map unit are 0.30, 0.30, 0.19, and 0.41 for regions one through four, respectively. These are somewhat higher, region for region, than the values for *In(1)* X^{c1}, *y*/rod X heterozygotes given above. Although the effect of the attachment of a Y chromosome (or a part of one) to one of the X's is perhaps not strictly comparable to the effect of the addition of a free Y chromosome, it is interesting to compare the present results with those of STURTEVANT and BEADLE, which are described above. While STURTEVANT and BEADLE found that the addition of a free Y chromosome had no effect on the exchange frequency within the inversion in one inversion heterozygote [*In(1)* Df (*bb*)], they found an increase in the exchange frequency within the inversion in other inversion heterozygotes. The only striking difference between *In(1)* Df (*bb*) and the other inversions used is that the former had no large subterminal heterochromatic segment, while the others did, as, of course, does *In(1)* X^{c1}, *y*.

A possible explanation for the observed depression of the frequency of crossing over in both the ring chromosomes and the *In(1)* X^{c1}, *y* rod chromosome is suggested by observations made by NOVITSKI and BRAVER (1953). They assumed that if the depression of crossing over in inversion heterozygotes were due to difficulties in euchromatic pairing, then chromosomes which paired in different ways would give different degrees of depression. They actually found, however, that the exchange frequency within the limits of the inversion in *In(1)* dl-49 heterozygotes is only about one quarter of the expected value in both attached-X and tandem metacentric compound chromosomes, which pair in quite different ways. They also found a high value relative to expectation between the proximal end of the inversion and the centromere, and a low value distal to the inversion, regardless of whether the inversion was heterozygous in normal rods, attached-X's, or tandem metacentric compounds. In order to explain these results, they postulated that "the synaptic tendency along the euchromatic length of the chromosome is very weak and ordinarily becomes manifest after homologous regions have been brought into proximity by stronger (but not necessarily specific) pairing centers located in the heterochromatic regions adjacent to the centromeres." As NOVITSKI and BRAVER pointed out, the postulated pairing "centers" were also proposed by GERSHENSON (1940) on genetic grounds, and by COOPER (1949, 1951) on cytological grounds. COOPER calls these pairing regions "collochores." LINDSLEY (1955) has demonstrated genetically that pairing *and exchange* between such regions on the X and the short arm of the Y

chromosome occur in Drosophila spermatogonia. Now as pointed out previously, *In(1)* X^{c1}, *y* has proximal heterochromatin at both ends of the euchromatic segment. Presumably, then, *In(1)* X^{c1}, *y* has collochores at both ends, and also more than the normal number. These would be expected to pair in meiosis, and probably in most mitoses. Observations of larval neuroblast mitoses show that the ends of *In(1)* X^{c1}, *y* do in fact have a tendency to associate so as to make it sometimes look like a ring chromosome. Meiosis in the *In(1)* X^{c1}, *y* male shows the same phenomenon. Both ends of the X chromosome are frequently associated with Y^s, and, commonly, the middle segments of the X's are asynapsed. (BENDER, Ph.D. thesis, unpub.)

If one assumes that this type of pairing also occurs in meiosis in the female, most of the observed crossover peculiarities of the *In(1)* X^{c1}, *y* chromosome can be explained by means of the hypothesis of NOVITSKI and BRAVER. One would expect that the heterochromatic pairing region of a normal X chromosome would pair at random with either end of the *In(1)* X^{c1}, *y* chromosome. If pairing were with the distal pairing region of *In(1)* X^{c1},*y*, then normal euchromatic pairing would follow; but if pairing was with the proximal pairing region of *In(1)* X^{c1}, *y*, euchromatic pairing would be more difficult, since the *bb* end of the euchromatin of *In(1)* X^{c1}, *y* would be closest to the *y* end of the euchromatin of the other chromosome. The chromosomes would then be able to pair only by forming an inversion loop. The increase in crossing over upon the addition of a Y^s arm to the distal end of *In(1) sc*^{8} might be explained if one assumed that pairing of Y^s with the proximal heterochromatin of *In(1)* X^{c1}, *y* was more likely than pairing with the distal heterochromatin of that chromosome. This might be expected if the proximal heterochromatin of *In(1)* X^{c1}, *y* has a greater length of what LINDSLEY (1955) calls "uninterrupted eucentric homology" than has the distal heterochromatin. As pointed out earlier, SCHULTZ and CATCHESIDE (1937) found that in X^{c1}, *y* the proximal heterochromatic duplication includes only a part of region 20 of the salivary gland chromosome.

While a number of assumptions are made in the above argument, it seems, at present, to be the best available explanation of the results of both the present and other experiments. The fact that the results can be explained in terms of the hypothesis of NOVITSKI and BRAVER, if not offering definite proof of that hypothesis, at least lends strength to it.

SUMMARY

The chromosome *In(1)* X^{c1}, *y* of *Drosophila melanogaster*, an inversion derived from the opening of the ring X^{c1}, *y*, shows the same peculiar double crossover values shown by X^{c1}, *y* and X^{c2}. When heterozygous with other inversions, it shows reduced values for single crossing over at the proximal end, and raised values at the distal end. In addition to the clearly confirmed effect of the proximity of the centromere on the frequency of crossing over almost all of the present experimental results are interpretable within and tend to confirm the hypothesis of heterochromatic pairing advanced by NOVITSKI and BRAVER (1953).

LITERATURE CITED

BEADLE, G. W. and A. H. STURTEVANT, 1935 X chromosome inversions and meiosis in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **21**: 384–390.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 552.

BRIDGES, C. B., and T. M. OLBRYCHT, 1926 . The multiple stock "Xple" and its use. Genetics **11**: 41–56.

COOPER, K., 1949 The cytogenetics of meiosis in Drosophila. Mitotic and meiotic autosomal chiasmata without crossing over in the male. J. Morphol. **84**: 81–121.

1951 Studies on spermatogenesis in Drosophila. Yrb. Am. Phil. Soc. 1951. pp. 146–147.

CREW, F. A. E., and R. LAMY, 1940 Spontaneous inverted exchange between X and Y in *Drosophila melanogaster*. J. Genet. **39**: 273–283.

DUBININ, N. P., 1933 Step-allelomorphism in *Drosophila melanogaster*. J. Genet. **27**: 443–464.

GERSHENSON, S., 1940 The nature of the so-called genetically inert parts of chromosomes. Vid. Akad. Nauk URSR **6**: 3–116.

GRUNEBERG, H., 1937 The position effect proved by a spontaneous reinversion of the X chromosome in *Drosophila melanogaster*. J. Genet. **34**: 169–189.

HUETTNER, A. F., 1924 Maturation and fertilization in *Drosophila melanogaster*. J. Morph. **39**: 249–265.

LINDSLEY, D. L., 1955 Spermatogonial exchange between the X and Y chromosomes of *Drosophila melanogaster*. Genetics **40**: 24–44.

MATHER, K., 1939 Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics **24**: 413–435.

MORGAN, L. V., 1926 Correlation between shape and behavior of a chromosome. Proc. Natl. Acad. Sci. U.S. **12**: 180–181.

1933 A closed X chromosome in *Drosophila melanogaster*. Genetics **18**: 250–283.

MULLER, H. J., 1930 Variations induced by X-rays in Drosophila. J. Genet. **22**: 299–334.

NOVITSKI, E., 1949 An inversion of the entire X chromosome. Drosophila Inf. Ser. **23**: 94–95.

1952 The genetic consequences of anaphase bridge formation in Drosophila. Genetics **37**: 270–287.

NOVITSKI, E., and G. BRAVER, 1954 An analysis of crossing over within a heterozygous inversion in *Drosophila melanogaster*. Genetics **39**: 197–209.

OFFERMANN, C. A., W. S. STONE, and H. J. MULLER, 1931 Causes of interregional differences in crossover frequency, studied in individuals homozygous for gene rearrangements. (Abstr.) Anat. Record **51**: 109.

OFFERMANN, C. A., and H. J. MULLER, 1933 Regional differences in crossing over as a function of the chromosome structure. Proc. 6th Intern. Cong. Genet. **2**: 143–145.

SCHULTZ, J., and D. G. CATCHESIDE, 1937 The nature of closed X chromosomes in *Drosophila melanogaster*. J. Genet. **35**: 315–320.

SIDOROV, B. P., 1936 The mutability of the "yellow" "achaete" and "scute" genes in the stock "scute^{8}." Biol. Zh. [Mosc.] **5**: 3–26.

SPENCER, W. P., and C. STERN, 1948 Experiment to test the validity of the linear r-dose/mutation frequency relation in Drosophila at low dosage. Genetics **33**: 43–74.

STERN, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**: 625–730.

STURTEVANT, A. H. and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

THE THEORY AND ANALYSIS OF DIALLEL CROSSES. II.

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1. *Introduction*

THE investigation of the properties of groups of homozygous lines by means of diallel crosses as proposed by HULL (1945) has subsequently been discussed in growing detail in a series of papers by JINKS and HAYMAN (1953 and later). With certain assumptions, the genetic variation present can be described in terms of the biometrical concepts of MATHER (1949) and accurate estimates obtained of the additive, dominance and environmental components. The distribution of genes in the parents is also revealed. The important result of these papers is that the diallel table of results can be tested (by the uniformity of W_r—V_r) for conformity to the biometrical model before applying the model to the description of the genetic variation present. The genetic systems controlling many characters do not fit this simple model and it is the purpose of this article to study more general genetic systems using new methods and more generations. This paper concerns parental, F_1 and F_2 data and contains an investigation of gene correlation in the parents together with some consideration of linkage and parental heterozygosity. Data from diallel crosses in maize and cotton will be discussed by kind permission of those who conducted the experiments. Reference to HAYMAN (1954b) is by section number prefixed by H.

2. *Analysis with F_2 data*

2.1. The n homozygous parent lines and their n^2-n hybrids were (H2.3) referred to jointly as the L_1 generation. The selfed offspring of this generation are called the L_2 generation. The means of families in this generation form the L_2 diallel table.

The assumptions or hypotheses on which our simple biometrical model is based are:—

(i). Diploid segregation,
(ii). Only environmental differences between reciprocal crosses,
(iii). Independent action of nonallelic genes,
and, in the parents:—
(iv). No multiple allelism,
(v). Homozygosity,
(vi). Genes independently distributed.

With these assumptions we can describe the genetic situation algebraically as

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in H2.3. The n homozygous parents have genotypes $\Theta_r = (\theta_{r1}, \theta_{r2}, \cdots \theta_{rk})$ where $r = 1, 2, \cdots\cdots n$, k is the number of genes controlling the measured character and $\theta_{ra} = 1$ for the positive homozygote AA, 0 for the heterozygote Aa and -1 for the negative homozygote aa. Their phenotypes are $p_r = \sum_{a=1}^{k} d_a \theta_{ra}$, environmental variation being ignored for the present. The offspring in the L_1 generation of the cross $\Theta_r \times \Theta_s$ have genotypes Θ_{rs} given by $\theta_{rs} = \frac{1}{2}(\theta_{ra} + \theta_{sa})$ and phenotypes $f_{rs} = \frac{1}{2} \sum_{a=1}^{k} [d_a(\theta_{ra} + \theta_{sa}) + h_a(1 - \theta_{ra}\theta_{sa})]$ so that $f_{rr} = p_r$. d_a and h_a measure additive and dominance deviations from the mid-homozygote at locus a.

The mean of the progeny of Θ_{rs} selfed is (H2.2(iv)) $g_{rs} = \frac{1}{2} \sum_a [2d_a\theta_{rsa} + h_a(1 - \theta^2_{rsa})] = \frac{1}{2} \sum_a [d_a(\theta_{ra} + \theta_{sa}) + \frac{1}{2} h_a(1 - \theta_{ra}\theta_{sa})]$ so that $g_{rr} = p_r$. Expectations in the L_2 diallel are the same as in the L_1 diallel with h_a replaced by $\frac{1}{2} h_a$.

2.2. Second degree statistics. The expectations of the second degree statistics of the L_1 diallel table were given in H2.4. Analogous statistics can be computed from the L_2 diallel table while the covariances between the two tables provide a further set of statistics. The expectations of all these statistics are listed in Table 1 in terms of genetical and environmental components. Table 1a contains just those statistics which depend on an individual array and Table 1b contains the averages of these statistics over the arrays together with the variance of the parents. Two of the statistics in Table 1a, W_{r12}' and W_{r21}', have the one average value, W_{CL12}.

Finally there is the expectation of the variance within the $r$sth L_2 family. Linkage is assumed absent. Then

$$V_{2(rs)L_2} = \tfrac{1}{4} \Sigma (d_a^2 + \tfrac{1}{2} h_a^2)(1 - \theta_{ra}\theta_{sa}) + E$$

The mean variance within L_2 families in the rth array

$$V_{2(r)L_2} = \tfrac{1}{4} \Sigma (d_a^2 + \tfrac{1}{2} h_a^2)(1 - w_a\theta_{ra}) + E$$

and the mean variance within all L_2 families

$$V_{2L_2} = \tfrac{1}{4} D + \tfrac{1}{8} H_1 + E$$

2.3. Description of components. The genetic components are

$$\begin{aligned}
D &= \Sigma d_a^2 (1 - w_a^2) \\
F_r &= 2 \Sigma d_a h_a \theta_{ra} (1 - w_a^2) \qquad (r = 1, 2, \cdots n) \\
F &= 2 \Sigma d_a h_a w_a (1 - w_a^2) \\
H_1 &= \Sigma h_a^2 (1 - w_a^2) \\
H_{r2} &= \Sigma h_a^2 (1 - w_a\theta_{ra})(1 - w_a^2) \qquad (r = 1, \cdots\cdots n) \\
H_2 &= \Sigma h_a^2 (1 - w_a^2)^2
\end{aligned}$$

F and H_2 are the respective means of F_r and H_{r2}. $w_a = u_a - v_a$ where u_a and v_a are the frequencies of AA and aa in the parents. D, F, H_1 and H_2 are overall measures of gene action: F_r and H_{r2} depend on the genetical constitution of the rth parent.

Only H_{r2} was not described in section H2. It has its least value when all $w_a\theta_{ra} = (u_a - v_a)\theta_{ra}$ are positive. Then θ_{ra} has the same sign as w_a, i.e. the rth parent contains the allele at locus a which is the more common in the parents. Parents whose

TABLE 1

Expectations in terms of genetical and environmental components

Generation	Statistic	Abbreviation	Meaning	Genetic expectation						Environmental expectation		
				D	F_r	F	H_1	H_{r2}	H_2	E_0	E_1	E_2
1a	$W_{01(r)L01}$	W_{r1}	$cov(p_s, f_{rs})$	½	−¼	..	..	..	..	$1/n$		
	L_1 $V_{1(r)L1}$	V_{r1}	$var f_{rs}$	¼	−¼	..	¼	..	..	$1/n$	$(n\text{-}1)/n$	
	$W_{01(r)L1}$	W_{r1}'	$cov(\bar{f}_s, f_{rs})$	¼	−⅛	−⅛	¼	−¼	..	$1/n^2$	$(n\text{-}2)/n^2$	
	L_1 $W_{1(r)L12}$	W_{r12}	$cov(f_{rs}, g_{rs})$	¼	−$\frac{3}{16}$	..	⅛	..	..	$1/n$		
	and $W_{10(r)L12}$	W_{r12}'	$cov(f_{rs}, \bar{g}_s)$	¼	−⅛	−$\frac{1}{16}$	⅛	−⅛	..	$1/n^2$		
	L_2 $W_{01(r)L12}$	W_{r21}'	$cov(\bar{f}_s, g_{rs})$	¼	−$\frac{1}{16}$	−⅛	⅛	−⅛	..	$1/n^2$		
	$W_{01(r)L02}$	W_{r2}	$cov(p_s, g_{rs})$	½	−⅛	..	..	..	..	$1/n$		
	L^2 $V_{1(r)L2}$	V_{r2}	$var g_{rs}$	¼	−⅛	..	$\frac{1}{16}$	..	..	$1/n$		$(n\text{-}1)/n$
	$W_{01(r)L2}$	W_{r2}'	$cov(\bar{g}_s, g_{rs})$	¼	−$\frac{1}{16}$	−$\frac{1}{16}$	$\frac{1}{16}$	−$\frac{1}{16}$	..	$1/n^2$		$(n\text{-}2)/n^2$
Parent	V_{0L0}		$var p_r$	1		..	..	..	..	1		
1b	W_{0L01}		$cov(p_s, \bar{f}_s)$	½	..	−¼	..	..	..	$1/n$		
	L_1 V_{1L1}			¼	..	−¼	¼	..	..	$1/n$	$(n\text{-}1)/n$	
	V_{0L1}		$var \bar{f}_s$	¼	..	−¼	¼	..	−¼	$1/n^2$	$(n\text{-}2)/n^2$	
	L_1 W_{1L12}			¼	..	−$\frac{3}{16}$	⅛	..	..	$1/n$		
	and											
	L_2 W_{0L12}		$cov(\bar{f}_s, \bar{g}_s)$	¼	..	−$\frac{3}{16}$	⅛	..	−⅛	$1/n^2$		
	W_{0L02}		$cov(p_s, \bar{g}_s)$	½	..	−⅛	..	..	..	$1/n$		
	L_2 V_{1L2}			¼	..	−⅛	$\frac{1}{16}$	..	..	$1/n$		$(n\text{-}1)/n$
	V_{0L2}		$var \bar{g}_s$	¼	..	−⅛	$\frac{1}{16}$	..	−$\frac{1}{16}$	$1/n^2$		$(n\text{-}2)/n^2$

genotype is the most common have the least value of H_{r2} while parents with uncommon genotypes have the greater values of H_{r2}. If the gene distribution in the parents is symmetrical (all $w_a = 0$) all genotypes are equally frequent and H_{r2} does not vary but if the gene distribution is asymmetrical H_{r2} separates the rare from the common genotypes amongst the parents. This paragraph only concerns genes exhibiting dominance.

E_0 is the average environmental variance of a parental entry in the diallel table and equals V_0/a_0 where V_0 is the mean variance between duplicate parental family means and a_0 is the number of duplicates of each parent line. E_1, for $\frac{1}{2}E'$ in H3.1, is the average environmental variance of the average of reciprocal and duplicate F_1 family means and equals V_1/a_1 where V_1 is the mean variance between reciprocal and duplicate F_1 family means and a_1 is the number of duplicates and reciprocals of each F_1 family. E_2 is similarly defined for F_2 family means from V_2 and a_2 but it contains a genetical component due to the sampling of segregation in the F_2 families. In some species, such as maize and cotton with which we will be concerned, each generation has a characteristic environmental variance so that we keep E_0, E_1 and E_2 separate though we could probably represent E_2 as a combination of E_0 and E_1 and a genetical sampling component. The environmental component E of the variance within F_2 families may also be assumed to be a combination of the mean variance within parental and the mean variance within F_1 families.

2.4. Description of gene distributions. The parental measurement p_r, with F_r and H_{r2}, together provide a general description of the genes in the parents. p_r is closely associated with the number of positive homozygotes in the rth parent, F_r with the number of dominant homozygotes, and H_{r2} with the number of rare homozygotes. The correlations between any pair of these three quantities reveal the interrelations of sign, dominance and rarity in the parental set of genes. We have

$$cov(p_r,F_r) = 2\,\Sigma d_a^2 h_a (1 - w_a^2)^2 > 0 \text{ if most } h_a > 0$$
$$cov(p_r,H_{r2}) = -\,\Sigma d_a h_a^2 w_a (1 - w_a^2)^2 > 0 \text{ if most } w_a < 0$$
$$cov(F_r,H_{r2}) = -\,\Sigma d_a h_a^3 w_a (1 - w_a^2)^3 > 0 \text{ if most } h_a w_a < 0$$

When a predominance of genes satisfies the far right inequalities the correlations are near unity, when a predominance satisfies the inverse inequalities the correlations are near negative unity while, if equal numbers of genes satisfy these inequalities and their converses, a zero correlation obtains. For instance, if positive alleles are more common than negative alleles in the parents then p_r and H_{r2} are negatively correlated while if dominance is mostly in the positive direction then p_r and F_r are positively correlated.

2.5. Estimation of components. This is not a straightforward problem and there are three questions to consider in its elucidation. Firstly, are linear or quadratic functions of d_a and h_a more informative about average gene action? As MATHER (1949) has shown, quadratic functions have a clear advantage and we, too, estimate D, H_1, etc. akin to MATHER's D and H.

Secondly, how should the components be estimated? In the expectations of

second degree statistics E_0, E_1 and E_2 have complicated coefficients in n. Even in an L_1 analysis with V_0 and V_1 assumed equal the standard errors of the components were awkward functions of n (Table H1). When L_2 and joint statistics are included such a solution becomes impractical. We suggest that E_0, E_1 and E_2 be estimated separately and as accurately as possible and subtracted from the observed statistics. The genetic components D, F, H_1 and H_2 and F_r and H_{r2} ($r = 1, 2, \text{———} n$) are then estimated by least squares.

Thirdly, what statistics should be used? We could employ only the ten statistics independent of r. Then the main components D, F, H_1 and H_2 could be estimated and there would be six degrees of freedom for error. Another course is to use the parental variance, V_{0L0}, the mean variance within F_2 families, V_{2L2}, and the $9n$ statistics excluding $V_{2(r)L2}$ which depend on r: all the genetic components can be estimated and $7n - 1$ * degrees of freedom remain for error. ($V_{2(r)L2}$ only supplies information about an unwieldy combination of additive and dominance variation). Since F_r and H_{r2} provide important information about the genetic system and since a larger number of degrees of freedom is desirable we follow the latter course except that we further exclude V_{0L0} and V_{2L2}.

The chief reason for excluding V_{0L0} and V_{2L2} is that a solution including them involves n in a complicated manner while their exclusion eliminates n entirely from the solution and leaves it as a simple factor in the standard errors. Another reason for excluding V_{0L0} is that it is correlated with every other statistic and so cannot supply much independent information. Further when n is large, the influence of V_{0L0}, if it is included, becomes negligible. Finally, when genotype-environment interactions are to be investigated by comparing gene action in diallel crosses grown in different seasons a useful preliminary is to standardize the scale of additive variation by dividing first and second degree statistics by $V_{0L0}{}^{1/2}$ and V_{0L0} respectively (ALLARD 1956). V_{2L2} is excluded on two further grounds. In a crop like maize it is more practical to measure total plot, than individual plant, yields. The variances of the yields of duplicate plots then contain only a small fraction of $\frac{1}{4}D + \frac{1}{8}H_1$ as a genetical sampling component and therefore little genetic information. Secondly, we have observed (2.2) that the environmental component of V_{2L2} is probably a combination of variance within parental and F_1 families. The proportions of combination can only be obtained from V_{2L2} itself with the knowledge of D and H_1 so that this statistic can provide no information about the genetic components.

Table 2 contains the least squares solution for the genetic components. D, H_1 and the mean components, F and H_2 are expressed in terms of the nine mean values of the $9n$ observed statistics. F_r and H_{r2} are expressed partly in terms of these mean statistics (first row of coefficients in each case) and partly in terms of the corresponding nine rth observed statistics (second row of coefficients). H_{r1} is to be ignored at present. The discrepancies between observed and expected

* That this number of degrees of freedom may in small diallels be greater than the total number n^2 of family means is a reflection of the approximate nature of the residual errors supplied by our least squares analysis of possibly highly correlated statistics.

TABLE 2

Solutions for genetic components

	W_{0L01} W_{r1}	V_{1L1} V_{r1}	V_{0L1} W_{r1}'	W_{1L12} W_{r12}	W_{0L12} W_{r}'	W_{0L12} W_{r21}'	W_{0L02} W_{r2}	V_{1L2} V_{r2}	V_{0L2} W_{r2}'
D	–1.8788	0.0500	0.1923	–0.4447	–0.3735	–0.3735	3.6465	0.6893	0.7248
F_r	–5.9625	1.7884	0.8785	–0.5964	–1.0514	–1.3714	8.0693	1.7191	1.3316
	–1.2800	–1.2800	0.1280	–0.9600	–0.2560	0.0640	–0.6400	–0.6400	–0.1280
F	–7.2425	0.5084	1.0065	–1.5564	–1.3074	–1.3074	7.4293	1.0791	1.2036
H_{r1}	–5.1043	2.8385	0.8270	0.1432	–0.8625	–1.0295	3.9626	1.0623	0.4759
	–0.6678	0.7235	0.0668	0.1948	–0.1336	0.0334	–0.3339	0.0139	–0.0668
H_1	–5.7721	3.5620	0.8938	0.3380	–0.9961	–0.9961	3.6287	1.0762	0.4091
H_{r2}	–0.5545	2.2559	0.0398	0.9893	–0.1187	0.0733	–0.4196	0.3898	–0.0683
	0.7680	0.7680	–2.6368	0.5760	–1.1264	–1.3184	0.3840	0.3840	–0.5632
H_2	0.2135	3.0239	–2.5970	1.5653	–1.2451	–1.2451	–0.0356	0.7738	–0.6315

values provide an estimate of error variance with $7n-3$ degrees of freedom ($9n-1$ linearly independent statistics less $2n+2$ linearly independent components). The sampling variances and covariances of the main estimated components are in Table 3. The factor $1/n$ in this table shows how the accuracy of the estimates of the genetic components increases with the size of the diallel cross.

2.6. Graphical representation. Our statistical investigation of the diallel cross describes as fully as possible the genes and their modes of action. However, tables of values of components and of correlation coefficients may not present information as vividly as diagrams. Graphical representation of the gene relations and properties is therefore a useful adjunct to the statistical analysis.

TABLE 3

Variance-covariance matrix

	D	F	H_1	H_2	
D	18.3435	44.2023	26.0589	–0.5692	$var_r F_r = 5.1200s^2$
F	44.2023	117.3748	75.2062	–1.9922	
H_1	26.0589	75.2062	63.3952	10.6726	$var_r H_{r2} = 12.0832s^2$
H_2	–0.5692	–1.9922	10.6726	22.4836	

With common multiplier s^2/n.

When only parental and F_1 observations are available the graph of W_{r1} against V_{r1} describes (H2.6) the dominance situation: it shows the variation of these statistics with F_{r2} the measure of relative dominance in the parents, and it shows the relationship between D and H_1.

The influence of H_{r2} may be exhibited in the graph of W'_{r1} against W_{r1}.

$$W_{r1} - 2W'_{r1} = \tfrac{1}{8}H_{r2} + \text{constant},$$

so that when the gene distributions in the parents are symmetrical the graph is a straight line of slope ½ through (W_{0L01}, V_{0L1}). With asymmetry the points are scattered about a line of slope ½ so as to indicate the rarity of the parental genotypes. The most common genotypes correspond to points above the line and uncommon genotypes to points below the line. If several rare mutations exist amongst the parents that parent containing the most of these mutations corresponds to the largest value of H_{r2} and the point furthest below the line.

These graphs may also be constructed from the L_2 table. For instance, the graph of W_{r2} against V_{r2} is a straight line of unit slope through (V_{1L2}, W_{0L02}) and describes the dominance phenomena in the same way as an L_1 graph with the degree of dominance reduced by a half. This reduction in scale can best be illustrated by plotting L_1 and L_2 points on the same diagram and drawing straight lines through the pairs of L_1 and L_2 points corresponding to the same parent. These lines radiate fanwise from the point where

$$V_r = \tfrac{1}{4}D - \tfrac{1}{8}H_1 + [E_0 - (n-1)(E_1 - 2E_2)]/n$$
$$W_r = \tfrac{1}{2}D + E_0/n$$

Evidently L_2 graphs mimic L_1 graphs on a smaller scale and furnish little new information. To take adavntage of F_2 data the two generations are better compared with each other than investigated separately by analogous procedures. We therefore introduce Figure 1 (see 6.6) where L_2 is plotted against L_1 for both W_r and V_r.

Since $W_{r1} - 2W_{r2} = -\tfrac{1}{2}D - E_0/n$

and $V_{r1} - 2V_{r2} = -\tfrac{1}{4}D + \tfrac{1}{8}H_1 - [E_0 - (n-1)(E_1 - 2E_2)]/n$

the graphs are straight lines of slope ½. Suppose the lines intercept the L_2 axis in A and B respectively. Then if the statistics have been corrected for E components estimates of D and H_1 may be read off the diagram:

$$D = 4OA$$
$$H_1 = 8OA - 16OB$$

The average degree of dominance $a = (H_1/D)^{\frac{1}{2}}$ is given by

$$a^2 = 2 - 4OB/OA$$

The W line lies above the origin: points with higher values correspond to more recessive parents and points with lower values to more dominant parents. The complete dominant is at

$$A_1 \equiv [\tfrac{1}{2}(1-a)D, \tfrac{1}{4}(2-a)D]$$

and the complete recessive at

$$A_2 \equiv [\tfrac{1}{2}(1+a)D, \tfrac{1}{4}(2+a)D]$$

If P is on the W line PA_2/A_1P is the ratio of the number of dominant to the number of recessive genes in the corresponding parent.

The V line falls halfway between the W line and the origin in the absence of dominance but generally lies below this position and passes below the origin when $a^2 > 2$. Points on the V line follow points on the W line, the complete dominant being at

$$B_1 \equiv [\tfrac{1}{4}(1-a)^2D, \tfrac{1}{16}(2-a)^2D]$$

and the complete recessive at

$$B_2 \equiv [\tfrac{1}{4}(1+a)^2D, \tfrac{1}{16}(2+a)^2D]$$

A_1B_1, A_2B_2 and the lines joining pairs of W_r and V_r points corresponding to the same parents are parallel and have slope $(1 - \tfrac{1}{4}a^2)/(1-a^2)$,

So far this new representation is merely a rearrangement of the old (V_r, W_r) graphs but it has two advantages. The first is that we are free to plot further comparisons of L_1 and L_2 statistics on the same diagram. For instance,

$$W_{r_1}' - 2W_{r_2}' = -\tfrac{1}{4}D + \tfrac{1}{8}H_1 - \tfrac{1}{8}H_{r_2} - [E_0 - (n-2)(E_1 - 2E_2)]/n^2$$

With symmetry of the gene distribution $H_{r_2} = H_1$ and this becomes a straight line of slope $\tfrac{1}{2}$. The W' line then lies halfway between the origin and the W line after correcting for E components and points on the W' line are in the same order as points on the W line but with half the spacing. In the general case of asymmetry the best fitting line of slope $\tfrac{1}{2}$ passes through (V_{0L_1}, V_{0L_2}) and cuts the L_2 axis at C. Then

$$H_2 = 16(OC - OB)$$

Points below the line correspond to common parents and points above it to uncommon parents.

A more complicated example is

$$(W_{r_1}' - W_{r_{12}}') - 2(W_{r_{21}}' - W_{r_2}') = \tfrac{1}{16}F + (n-2)(E_1 + 2E_2)/n^2$$

$W_{r_1} - W_{r_{12}}'$ is the covariance between the rth L_1 array and the differences of L_1 and L_2 array means while $W_{r_{21}}' - W_{r_2}'$ is the similar covariance involving the rth L_2 array. Their graph is a straight line of slope $\tfrac{1}{2}$ and since both differences vary with $-H_{r_2}$ greater values correspond to common genotypes and lesser values to rare genotypes. If the line intercepts the L_2 axis at D then

$$F = -32OD.$$

The second and most important advantage of this new diagram is that the W, V and W' lines are disturbed by characteristically different failures of the hypotheses (section 4).

3. *Testing the hypotheses*

As a preliminary to the estimation of components or the description of the genes in the parents the hypotheses of 2.1 must be shown to hold for the data under consideration. The first and second degree statistics provide separate tests.

3.1. First degree statistics. HAYMAN (1957) extends MATHER's (1949) C test of epistasis in a single cross and its F_1 and F_2 families to a complete diallel cross.

A single χ^2 compares with E_0, E_1 and E_2 the squared and summed deviations of all the parental, F_1 and F_2 family means from their expectations on a genetic model exhibiting additive and dominance variation.

3.2. Second degree statistics. As consequences of the hypotheses various differences between second degree statistics involving r are independent of r. In H2.6 we mentioned the constancy of $W_{r_1} - V_{r_1}$ and in 2.6 $W_{r_2} - V_{r_2}$, $W_{r_1} - 2W_{r_2}$ and $V_{r_1} - 2V_{r_2}$. In a properly designed experiment with replicate blocks we can analyse a table of values of any one of these quantities and significant variation with r indicates failure of one or more of the hypotheses. Since the nine second degree statistics which vary with r depend on the two variable parameters, F_r and H_{r_2}, there must be altogether seven independent comparisons amongst these statistics each providing a separate test of the validity of the hypotheses. We have seen that some of these comparisons may be represented graphically. They can all be combined into one analysis of variance. If the sum of squares of deviations of observed from expected statistics after fitting D, H_1 and F_r and H_{r_2} ($r = 1, \ldots n$) to the block totals of the $9n$ statistics is R_1 with $7n-3$ degrees of freedom and if the sum of squares after fitting these components separately to each of the b blocks is R_2 then $R_2 - R_1$ provides an error variance for R_1 with $(b-1)(7n-3)$ degrees of freedom.

We must not be tempted to infer regression tests of the hypotheses from the diagrammatic representations of the relations between the statistics. If in a certain genetical situation the expectations of two second degree statistics, x_r and y_r, both contain the component F_r there is a linear relation, $y_r - bx_r = \text{constant}$, between them. In a more complicated genetical situation $y_r - bx_r$ may not be constant and the test of failure of the original hypotheses is the analysis of variance of $y_r - bx_r$. In the original situation the graph of x_r against y_r is a straight line of slope b. When the hypotheses fail this graph is disturbed; either its position or slope may be changed or it may be transformed into a curve or a scatter of points. To test fully the hypotheses in terms of the graphical representation all these possibilities must be taken into account: any simple test such as a regression test of the deviation of the slope from b is incomplete and insensitive. Three illustrative failures of the hypotheses which hardly alter the slope of the graph of W_{r_1} against V_{r_1} may be cited. Firstly there is a case of failure of hypothesis (iii): a low intensity of duplicate gene action curves the graph slightly (H4.5). There is a similar effect if the gene distributions in the parents are correlated (H4.4 and 4.3). Thirdly, if the parents are partially heterozygous the (V_{r_1}, W_{r_1}) graph is a scatter about a line of unit slope (H4.3 and 4.4). But more important than any objection on the grounds of insensitivity is the absence of a causal relation between the variables under consideration here so that regression is strictly inapplicable. Now if regressions "either way" conformed this absence might be ignored in practice. Of 21 (V_r, W_r) graphs mostly from *Nicotiana rustica* experiments ten reveal failure of the hypotheses at the .05 probability level solely through regression of W_r on V_r, two solely through regression of V_r on W_r and one through both regressions. The random expectation for the last group is 1.6

so that agreement between the two tests is fortuitous. The result of a regression test is therefore ambiguous. A modified regression test which avoids this ambiguity is described in H4.1 but is to be used only when lack of replication renders the analysis of variance impossible.

3.3. Comparison of tests. These two tests are not concerned with the first two hypotheses. The first hypothesis is verified by nonstatistical means and we avoid the second by averaging reciprocals. The χ^2 test is basically MATHER's (1949) scaling test $C = 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2$ applied to each cross of the diallel in turn and then accumulated over the complete set of crosses with due allowance for recurrent parentage and the varying accuracy of measurement of each generation. MATHER's C is a test of individual crosses and so is zero not only in our model but also when any of the last three hypotheses fail: our χ^2 detects only nonallelic interaction (epistasis) and cannot reveal either multiple allelism, heterozygosity or gene correlation in the parents. On the other hand the test of second degree statistics may reveal failure of any one or more of these four hypotheses. Another point of comparison between the two tests is that whereas the latter is somewhat unsatisfactory statistically the former is as accurate as E_0, E_1 and E_2. Further whereas the latter can only be used with a replicated experiment the former can be applied to a single block as long as it contains duplicates.

4. Analysis when the hypotheses fail

4.1. When failure of the hypotheses has been demonstrated, the simple theory of section 2 is no longer applicable: a more complex genetical system must be postulated and new parameters introduced to represent it. When only L_1 data were available there were not enough statistics to estimate these new parameters and a qualitative estimate of the bias caused by their presence was all that was possible (H4.1). With data from another generation we should be able to investigate some of these more complex genetic systems. Here we consider linkage and also failures of hypotheses (v) and (vi), that is to say, heterozygosity in, and correlation between, the genes in the parents. The important topic of epistasis must wait until later although HAYMAN (1957) has given it some consideration.

4.2. Linkage. F_2 is the only segregating generation in this diallel experiment and the variances within F_2 families are the only statistics disturbed by linkage. However, since these variances often provide no information about second degree genetic components even in the absence of linkage (2.5) it is not possible to test for linkage in the analysis of second degree statistics. A higher degree and therefore less reliable test can be constructed from the diallel table of F_2 family variances. In the absence of linkage.

$$\underset{r}{var}V_{2(rs)L_2} = \tfrac{1}{16}\Sigma(d_a^2 + \tfrac{1}{2}h_a^2)^2(1 - w_a^2),$$

i.e. the variances of the arrays of this table should be constant. It can be shown that with linkage these variances may not be constant.

4.3. Correlated gene distributions. The extension of the model to accommodate gene correlation is straightforward. Previously the genes at different loci were uncorrelated so that $cov(\theta_{ra},\theta_{rb}) = 0$ $(a \neq b)$. Now, while $var\theta_{ra}$ is still $1—w^2{}_a$

suppose that $cov(\theta_{ra},\theta_{rb}) = c_{ab}$ $(a \neq b)$. The expectations in Table 1 hold except that in 1a H_1 is replaced by H_{r_1} and the genetic components are redefined as follows:—

$$
\begin{aligned}
D &= \Sigma d_a^2 + \Sigma_{a \neq b} d_a d_b c_{ab} \\
F_r &= 2\Sigma d_a h_a \theta_{ra}(1 - w_a^2) + 2\Sigma d_a h_a \theta_{rb} c_{ab} \\
F &= 2\Sigma d_a h_a w_a (1 - w_a^2) + 2\Sigma d_a h_a w_b c_{ab} \\
H_{r_1} &= \Sigma h_a^2 (1 - w_a^2) + \Sigma h_a h_b \theta_{ra} \theta_{rb} c_{ab} \\
H_1 &= \Sigma h_a^2 (1 - w_a^2) + \Sigma h_a h_b (w_a w_b + c_{ab}) c_{ab} \\
H_{r_2} &= \Sigma h_a^2 (1 - w_a \theta_{ra})(1 - w_a^2) + \Sigma h_a h_b (\theta_{ra} - w_a) \theta_{rb} c_{ab} \\
H_2 &= \Sigma h_a^2 (1 - w_a^2)^2 + \Sigma h_a h_b c_{ab}^2
\end{aligned}
$$

H_1 is now the mean of the H_{r_1}. The influence of gene correlation on our statistics is analogous to the influence of linkage on statistics derived from variation within segregating generations. MATHER'S (1949) D and H change their form with linkage in much the same way as our D, F, H_1 and H_2 change with corelation. Indeed, if the parents of the diallel cross originated by inbreeding from a single F_2 family, correlation between the genes might well be a relic of linkage in the original family.

Table 2 contains solutions for the components. The variance-covariance matrix for the main components is still Table 3.

The new component H_{r_1} reveals the type of gene correlation present. With *association* (genes of like effect together in excess in the parents or all $c_{ab} > 0$) maximum values of H_{r_1} correspond to complete dominant or complete recessive parents and minimum values of H_{r_1} to parents containing a mixture of dominant and recessive genes. With *dispersion* (genes in opposition in excess or most $c_{ab} < 0$) the opposite situation holds. Since $V_{r_1} - 2V_{r_2}$ varies with H_{r_1} (2.6), the V graph is convex upwards with association and convex downwards with dispersion. The W graph is still linear and of slope ½ when gene correlation is present. When the gene distribution is symmetrical $H_{r_1} = H_{r_2}$ so that $W_{r_1}' - 2W_{r_2}'$ is constant (2.6), the W' graph is a straight line of slope ½. This graph is an indicator of gene asymmetry whether or not gene correlation is present. The influence of gene correlation on the main components D, F, H_1 and H_2 has been discussed in H4.4. We repeat here that the measure of degree of dominance $(H_1/D)^{½}$ may be either increased or decreased; the particular combination of dispersion and unidirectional dominance inflates H_1/D seriously and may easily turn partial dominance into apparent overdominance.

Gene correlation is not detected by the χ^2 test (3.3). The $3n+1$ correlation components fitted to the block means of the $9n-1$ independent second degree statistics leave a sum of squares R_1' with $6n - 2$ degrees of freedom. If this is significant against its block interaction one or more of the remaining hypotheses (iii), (iv) or (v) must fail. If R_1' is not significant, significance of $R_1 - R_1'$ probably indicates gene correlation.

The importance of utilizing the statistics associated with individual arrays and not just statistics averaged over arrays in tests of second degree statistics is now

more firmly established. The expectations of mean second degree statistics can be expressed in terms of some D, F, H_1 and H_2 whether or not gene correlation is postulated and that D, F, H_1 and H_2 should have different structures in these two situations is irrelevant to their estimation or to their goodness of fit to the observed mean statistics (cf. JINKS 1956; KEMPTHORNE 1956). Only by examining the V graph and possibly the W' graph or by utilizing all $9n$ observed statistics in the goodness of fit test can gene correlation be detected.

4.4. Partly inbred parents. If there is some residual heterozygosity in the parents the expectations in terms of components of the second degree statistics are the same as in the previous subsection. Only the structure of the components is changed and it now involves the degree of inbreeding f_a at each locus in the parents (WRIGHT 1923) instead of the covariances c_{ab}.

The W graph is still a straight line of slope ½: maximum values of W correspond to parents homozygous for recessive genes, minimum values to parents homozygous for dominant genes and intermediate values to parents which are either heterozygous or homozygous for a mixture of dominant and recessive genes. The V graph is a scatter of points about a straight line of slope ½, points below the line corresponding to more homozygous parents and points above to more heterozygous parents. The (V_{r_1}, W_{r_1}) graph has similar properties (H4.3) and DICKINSON and JINKS (1956) give illustrative graphs for two symmetrical models. The behaviour of the W' graph (2.6) is virtually unaffected by the level of inbreeding in the parents: it remains an indicator of gene asymmetry and of the rarity of the parental genotypes.

The estimates of the components and their errors are obtained from Tables 2 and 3 and any test of the second degree statistics for gene correlation is a test for parental heterozygosity. The χ^2 test is not disturbed by parental heterozygosity.

4.5. Discussion. We can test the L_1 generation for conformity to the simple genetic system of 2.1 but it does not contain enough information to distinguish between different failures of the hypotheses defining this system. The addition of the F_2 generation supplements this information enough to separate the various more complex genetic systems in some circumstances.

The χ^2 (3.2) tests for epistasis alone. Failure of second degree statistics to conform to the model which accommodates gene correlation or heterozygosity in the parents indicates either epistasis or multiple allelism or both. Since the W graph is disturbed by these two factors alone a simpler and quicker, though less sensitive, test is the analysis of variance of $W_{r_1} - 2W_{r_2}$. Significance here, coupled with nonsignificance of the χ^2, suggests multiple allelism. Finally, if the second degree statistics do not fit the simple system of 2.1, any one or more of the last four hypotheses may fail. If at the same time the previous test suggests that the genes all have two alleles and are not epistatic then the genes in the parents are probably correlated or the parents are only partly inbred or both. Unfortunately the identity in terms of components of variation of systems containing correlated genes or heterozygous parents precludes any further separation of failures of the

hypotheses by means of second degree statistics; higher order statistics would have to be invoked. Some progress may be possible from the visual examination of the scatter of points in the V graph, this being equivalent to investigating fourth degree statistics. Heterozygosity causes a scatter about a straight line of slope ½. Simple correlations curve this line but mixed correlations would create a combination of curves indistinguishable from a heterozygosity graph. In practice confusion is unlikely to occur because only a small degree of heterozygosity could remain undetected in the parents. To summarize, we can always detect epistasis, we can detect multiple allelism in the absence of epistasis and lastly, when both these factors are absent, gene correlation may be exposed.

Having largely accomplished the separation of the possible genetic systems we turn to their quantitative description. Now the simple genetic system is described by our analysis in no more detail than by the analysis of L_1 data alone though the accuracy of our description is considerably greater. Unfortunately the situation is similar when the genes are correlated or partly heterozygous in the parents; the addition of the F_2 generation adds no more information to that in the L_1 generation alone about the parameters in the mathematical description of these systems. All that can be done is to describe qualitatively the unmeasurable disturbances caused by these factors to the simple analysis, e.g., H_1/D is inflated by one type of correlation or gene asymmetry is exaggerated if the parents are not quite inbred.

We should emphasize here that, throughout this article, we are investigating a given set of inbred lines: the D, H_1, etc., from an experiment characterize just those lines involved in the experiment. We do not try, nor intend to try, to infer properties of any population from which these lines might have been selected. Furthermore, the standard errors of our genetical components arise from sampling the environment and also from sampling the segregation in the F_2 families. These errors do not measure possible variation due to sampling the inbred lines from any hypothetical genetical population pool.

5. *Experimental design*

5.1. Our methods of analysis suggest general principles for the design of diallel cross experiments. The test of conformity of second degree statistics to the biometrical model set up in 2.1 requires replication of the experiment to provide a source of error for the statistics. The χ^2 test of linear statistics requires parental, F_1 and F_2 families together with a source of E_0, E_1 and E_2 in each block. Evidently the experiment should be replicated and each replicate should contain all these families duplicated.

5.2. Number of replications. The number of replicates can be inferred from the standard errors of the second degree genetic components in the light of NELDER's (1953) suggestion that these are better estimated from replicate values than from the residuum of a least square estimation. We take as criterion that genetic variation must be shown to exist or, in other words, that the additive component of genetic variation D must be significantly above zero. NELDER would presumably test the deviation of the mean value of $\hat{D}$ from zero by a one-sided Student's t

using the variance of separate replicate values to provide the standard error. NELDER's (1953) figures for MATHER and PHILIP's five-replicate barley experiment (MATHER 1949) lead to a probability of .01–.001 that the average D is zero. We find the same probability for the maize and cotton experiments which each contain four replicates. JINKS' (1954, 1956) *Nicotiana rustica* experiments used two replicates. His measurements of height and flowering time for three years provide six tests of deviation of $\bar{D}$ from zero of which one is significant at P = .01–.001 and two at P = .05–.01. The criterion suggests that about four replicates are necessary.

5.3. *Number of duplicate families.* Both the χ^2 test of epistasis and the method of estimating the genetic components require measures of the environmental components in each block. E_0, E_1 and E_2 should be estimated separately from the variation amongst duplicate parental, F_1 and F_2 families and not as a common E from the pool of this variation because simplifying assumptions, such as $V_0 = V_1$, are incorrect in some species. Table 4 shows that $V_0 \neq V_1$ in maize or cotton.

TABLE 4

BARTLETT's *test of homogeneity of variances of duplicate families*

		χ^2	D.F.	Prob.	Mean variance	D.F.
Maize	Between parents	10.78	9	n.s.	$V_0 = 294 = E_0$	30
	Between F_1 families	58.44	44	n.s.	$V_1 = 2111 = E_1$	135
	Between F_2 families	45.65	44	n.s.	$V_2 = 866 = E_2$	135
	Total within generations	114.87	97	n.s.		
	Between generations	48.64	2	<.001		
Cotton	Between parents	13.78	6	.05–.02	$V_0 = .037 = 6E_0$	161
	Between F_1 families	15.95	20	n.s.	$V_1 = .107 = E_1$	63
	Between F_2 families	19.61	20	n.s.	$V_2 = .132 = E_2$	63
	Total within generations	49.34	46	n.s.		
	Between generations	48.97	2	<.001		

We can compute the number of duplicate families required to give sufficiently accurate environmental corrections to the second degree statistics. Consider first the variance V_0 between duplicate parental plots. If a_0 is the number of duplicates per parent line in each block then $E_0 = V_0/a_0$. The total number of parental plots in a block is na_0 and the number of degrees of freedom of V_0 is $n(a_0 - 1)$. The greatest correction involving E_0 is E_0/n (Table 1). Since a variance V with f degrees of freedom has a standard deviation of $V/(2f)^{\frac{1}{2}}$

$$sd(E_0/n) = V_0/(2n^3a_0^2(a_0 - 1))^{\frac{1}{2}}$$

Suppose that the error in the environmental correction to the statistic is to be less than a fraction p of the additive component of that statistic. The minimum additive component in a statistic containing E_0/n is $\frac{1}{4}D$ so that we put

$$p = sd(E_0/n)\,\tfrac{1}{4}D$$

Hence $a_0^2(a_0 - 1) = 8V_0^2/n^3p^2D^2$ and it is easy to find the integral value of $a_0 \geqslant 2$ which makes the left side of this equation not less than the right side. In a similar way the number of duplicates of F_1 families is given by

$$a_1^2(a_1 - 1) = 16(n - 1)V_1^2/n^3p^2D^2$$

and the number of duplicates of F_2 families by

$$a_2^2(a_2 - 1) = 16(n - 1)V_2^2/n^3p^2D^2$$

The formula of the χ^2 test of epistasis (HAYMAN, 1957) contains $8E_2 + 2E_1$ as a factor so that the influence of errors in the environmental components is greater here than in the second degree statistics. However, high accuracy in χ^2 is unnecessary and the level of accuracy of E_0, E_1 and E_2 suggested above is probably quite suitable.

6. Maize and cotton yield

6.1. DR. G. F. SPRAGUE has supplied the yields of a maize diallel cross. This contained ten inbred lines and all possible derived F_1 and F_2 families. One plot of each family was grown in each of four replications. KINMAN and SPRAGUE (1945) have already considered these yields but corrected for moisture content so that their results may not be comparable with ours. DR. J. H. TURNER has supplied the yields of upland cotton from a similar experiment involving seven lines. His parents, however, had six duplicates in each block. He discussed these yields in 1953 in terms of analysis of variance.

We illustrate our theory and analysis with this data. Of course, these experiments were not designed to suit our methods and where they are inadequate we arbitrarily supply the missing information. This is not in any way intended as criticism of these experiments; in exemplifying our methods with this data we are using them for a purpose for which the experiments were not intended but we prefer this to constructing completely synthetic sets of results as illustrations. Further we do reach some important conclusions about genetic control of maize yield.

6.2. Scale and environmental variances. The first step in any biometrical analysis is the minimization of genotype-environment interaction to render genetic and environmental effects separable. The criterion for the ideal situation is the homogeneity of the variances between duplicate plots of each family in each block of the experiment. Now in the maize experiment only one plot of each family was grown in each replicate. To overcome this difficulty we here use the variances between replicate plots. In the cotton experiment the parents were duplicated but alone do not warrant special consideration and we pool replicates and duplicates to provide single estimates of the environmental variances of each parent.

The main section of Table 4 contains BARTLETT's χ^2 test for homogeneity of variances both between families within generations and between generations. Evidently the ideal of uniform variances is met only in part. In any one generation the families seem to be equally variable but the level of variability differs markedly from generation to generation. We might eliminate the differences be-

tween generations by a change of scale (WRIGHT 1952) but would probably simultaneously generate differences within generations. We let yield itself stand as scale and accommodate the differences in environmental variability between generations in the analysis. The homogeneity of F_2 family variances suggests that the genetic sampling fraction of E_2 is small. The final columns of Table 4 contain the mean variances of replicates (and duplicates) and their degrees of freedom in the two species. With the exception of that relating to the cotton parents these figures will be used here for E_0, E_1 and E_2 in each block. E_0 for cotton is one sixth of the value in Table 4 because six duplicates of each parental family were grown in each block. In the ideal experiment E_0, E_1 and E_2 would be computed separately for each block from the duplicates in that block.

We may note that Table 4 supplies two examples of crossbreeding species in which F_1 families are less stable than their inbred parents. LERNER (1954, Table 18) gives a contrary result for maize but using the coefficient of variation to measure variability. The latter measure is equivalent to the standard deviation on a logarithmic scale so that LERNER's and our conclusions are in effect drawn from different characters.

6.3. χ^2 test of epistasis. This χ^2 is calculated for each block and for the block totals. When block totals are considered the environmental variance of, for example, the parents is the sum of E_0 for each block, or $4E_0$ in our examples. Table 5

TABLE 5

Test of family means for epistasis

	Maize			Cotton		
	χ^2	D.F.	Prob.	χ^2	D.F.	Prob.
Epistasis	100.82	45	<.001	58.79	21	<.001
Blocks × epistasis	138.46	135	n.s.	33.46	63	n.s.

is the analysis of χ^2. Evidently epistasis is important in both species. The χ^2 item, blocks × epistasis, though normally of import, is to be ignored here because variation between the blocks has been absorbed into the present E_0, E_1 and E_2 and is therefore self-compensating in this item.

Table 6 contains the yields of each maize family summed over the four blocks together with their deviations from the nearest epistasis-free yields, calculated by the method of HAYMAN (1957). Parental values lie on, F_1 above, and F_2 below, the leading diagonal, and deviations significant at five percent are asterisked. Corresponding F_1 and F_2 deviations are proportional and are significant or not together and we may say that a significant pair of F_1 and F_2 deviations indicates an epistatic cross. Five parents and eight crosses are epistatic, the crosses exhibiting a complementary type of gene action (F_2 nearer to the midparent than the F_1 would imply). On the average the epistatic and nonepistatic crosses exhibit much the same dominance—or heterosis, since all the F_1 yields lie outside their parental yields. HAYMAN (1957) finds that in maize yield cor-

TABLE 6

Yields of maize families

Parents	1 HY	2 R46	3 B2	4 WF9	5 38/11	6 OHO7	7 OHO4	8 WV7	9 K159	10 CI14	
											F_1
HY	560	1264	1296	1092	1394	1161	1409	1226	1408	987	
	–7	–110*	–80	31	51	64	–51	–18	–6	28	
R46	768	603	1325	1313	1477	1181	1193	1073	1383	1385	
	90*	–31*	18	–34	–46	69	37	–38	–79	–240*	
B2	822	970	609	1571	1525	1690	1416	1420	1460	987	
	65	–15	–46*	–175*	–42	–166*	–73	–120*	29	–15	
WF9	828	857	798	425	1474	1393	1340	1236	1239	1155	
	–26	28	143*	–15	–9	–59	46	–43	51	–19	
38/11	1007	925	951	931	434	1446	1145	1633	1567	1244	
	–42	37	35	8	–19	21	–35	–86	14	–131*	
OHO7	916	937	879	833	967	460	1439	1421	1403	1246	
	–53	–57	136*	49	–17	–10	89	–70	–74	–12	
OHO4	840	852	817	897	694	1013	260	875	1650	1170	
	42	–30	60	–38	29	–73	–4	13	–133*	53	
WV7	801	703	766	738	880	805	592	340	1351	807	
	15	31	99*	35	71	57	–10	–45*	–190*	–58	
K159	1042	938	1116	997	1113	923	921	706	859	1315	
	5	64	–24	–42	–12	61	109*	156*	–30*	–25	
CI14	672	522	617	658	556	722	722	403	835	41	
	–23	196*	12	15	107*	10	–44	48	20	–30*	
F_2											

rected for moisture content complementary epistasis is present and is associated with greater heterosis while in cotton yield complementary epistasis and the lower levels of heterosis go together. The result for the corrected maize yield can be ascribed to selection for heterosis having proceeded long enough to associate the desirable complementary genes with high levels of dominance. In the raw maize yield and especially in cotton yield it seems as if an increase in heterosis could be achieved by transferring complementary genes into association with genes exhibiting the higher degree of dominance.

Nonepistatic maize diallels can be selected from Table 6. The largest is obtained by eliminating arrays R46, B2, K159 and CL14. Two nonepistatic five-line diallels can be selected from the cotton data.

6.4. Test of second degree statistics. In each block the L_1 and L_2 diallel tables supply the statistics of Table 1. These are corrected for E_0, E_1 and E_2 and components of genetic variation calculated by means of Table 2 from the statistics of each block and also from the totals of the statistics of each block. Table 7 is the

TABLE 7

Analysis of variance of second degree statistics

	Maize			Nonepistatic maize			Cotton		
	Var.	D.F.	Prob.	Var.	D.F.	Prob.	Var.	D.F.	Prob.
Interaction and multiple allelism	850	58	<.001	1097	34	.20–.10	.0203	40	<.001
Interaction, multiple allelism, parental heterozygosity and gene correlation	480	9	.05–.01	1069	5	.10–.05	.0113	6	<.001
Blocks × components	1750	66		3124	42		.0206	48	
Blocks × i. and m. a.	382	174		815	102		.0043	120	
Blocks × i., m.a., p.h. and g.c.	160	27		389	15		.0012	18	

analysis of variance of residuals after first fitting the components of Table 1 (the simple model) and then also fitting H_{r_1} instead of H_1 (allowing for parental heterozygosity and gene correlation). The item, blocks × components, makes the block interaction items up to the full sum of squares between replicates. The block interactions are heterogeneous—probably a consequence of the nonnormality of second degree statistics—so that each main item is tested against its own block interaction. The high blocks × components item may be due to this nonnormality but more probably to our use of the same E_0, E_1 and E_2 in each block. The maize and cotton analyses are similar. Both confirm the χ^2 detection of epistasis and there may also be some multiple allelism. The significance of the other main item cannot be interpreted to indicate parental heterozygosity and/or gene correlation because the undoubtedly present epistasis and possible multiple allelism can also inflate this item.

Table 7 also contains the analysis of variance of second degree statistics from the maize diallel which is nonepistatic according to the χ^2 test. Neither main item is significant so that there is no suggestion that this reduced six-line diallel departs from the simple model of 2.1. This attainment of the simple model on removal of epistasis is not usual. HAYMAN (1957), considering only $W_{r_1} - V_{r_1}$, found that heterogeneity of this difference was eliminated by the removal of epistasis in only two out of six cases. One of the two nonepistatic five-line cotton diallels fits the simple model according to the test of $W_{r_1} - V_{r_1}$ and the other does not. We will not test the cotton data further because of the work involved and will concentrate mainly on the maize data.

6.5. Genetic components. Table 8 contains the mean genetic and environmental components from the four maize replications. These have been computed both from the complete data and from the nonepistatic six-line diallels. The standard errors of the main genetic components are derived from Table 2 with s^2 as the mean of all the variance components in the appropriate section of Table 7. These uniform standard errors are not strictly applicable to variance components the errors of which are related to their magnitude and the fourth column of Table 8 contains standard errors with three degrees of freedom calculated from the replicate values according to NELDER's (1953) method. Errors for E_0, E_1 and E_2 would

TABLE 8

Components of variation in maize yield

	Main components	Standard errors		Components which vary with r									
		Table 6	Nelder	HY	R46	B2	WF9	38/11	OHO7	OHO4	WV7	K159	CI14
Complete data	E_0 294	....	....	....	....	....	....	....	....	....	....	....	....
	E_1 2111	....	....	....	....	....	....	....	....	....	....	....	....
	E_2 866	....	....	....	....	....	....	....	....	....	....	....	....
	D 2661	574	320	....	....	....	....	....	....	....	....	....	....
	F 971	1452	728	6202	8070	2275	3757	–2938	3814	–9159	–5037	11826	–9104
	H_1 21182	1067	2015	19806	20096	21496	21004	21622	22059	22129	21824	20460	21325
	H_2 20436	635	2080	17157	17079	20328	19630	26420	21963	24821	18943	17474	20550
	H_1–D 18521	729	2194	....	....	....	....	....	....	....	....	....	....
	$(H_1/D)^{1/2}$ 2.82	....	....	....	....	....	....	....	....	....	....	....	....
Six-line diallel	D 664	1107	86	....	....	....	....	....	....	....	....	....	....
	F 270	2800	934	11512	....	....	4266	–1961	4886	–11491	–5591	....	....
	H_1 37470	2058	2278	35211	....	....	36993	38485	37809	38104	38219	....	....
	H_2 37181	1225	2398	31640	....	....	34916	43111	39080	39081	35259	....	....
	H_1–D 36806	1406	2269	....	....	....	....	....	....	....	....	....	....
	$(H_1/D)^{1/2}$ 7.51	....	....	....	....	....	....	....	....	....	....	....	....

also be obtained from this source but this is not possible in our case. The only important difference is that $\hat{D}$ in the six-line diallels here deviates significantly from zero. Otherwise both sets of standard errors agree that $\hat{F}$ is not significantly different from zero while $\hat{H}_1$, $\hat{H}_2$ and $\hat{H}_1-\hat{D}$ are all greater than zero. Before discussing the values of the components it is of interest to check whether ignoring the variance of the parents, V_{0L0}, is likely to have upset our estimates. The values of $V_{0L0}-E_0$ are 3019 and 616 which agree with the tabulated estimates of D.

The second value of $\hat{D}$ in Table 8 is much less than the first: the parents in the six-line diallel differ either by fewer genes than the complete set of ten parents or by genes grouped more in dispersion (4.3). The degree of dominance $(\hat{H}_1/\hat{D})^{\frac{1}{2}}$ in the six-line diallel indicates a high degree of apparent overdominance. HAYMAN (1957) also finds apparent overdominance ($(\hat{H}_1/\hat{D})^{\frac{1}{2}}=1.42$ and 2.53) in nonepistatic cotton yield. Whether these are due to genuine single-gene overdominance or to combinations of favourable dominants and unfavourable recessives in the parents cannot be decided without reference to the variation within families in segregating generations—and we have no useful measure of this variation in these two examples. $(\hat{H}_1/\hat{D})^{\frac{1}{2}}$ can also be computed from the epistatic diallels but then it is not certain what it measures and it must be called the apparent degree of dominance. Now $\hat{H}_1$ is significantly greater than $\hat{D}$ here too and since the standard error of $\hat{H}_1-\hat{D}$ includes deviations from the simple genetic model as well as differences between duplicates and although epistatic parameters would not be orthogonal to additive and dominance parameters in our second degree statistics we have a strong presumption of apparent dominance in the ten lines taken together. On selecting a nonepistatic subdiallel from the maize data the apparent degree of dominance is more than doubled and a similar though smaller rise occurs with the cotton data. The more comprehensive examination by HAYMAN (1957) of this change in apparent degree of dominance shows that it has no obvious interpretation except as a sampling effect. The remaining main component $\hat{F}$ has a negligible value so that either gene asymmetry favours positive alleles at as many loci in the parents as it favours negative alleles or else dominance is as often positive as negative.

The variability over r of $\hat{F}_r$ and $\hat{H}_{r2}$ in Table 8 is largely genetic for it is much greater than the sampling variation given by Table 3. The variabilities of the three quantities $\hat{p}_r$, $\hat{F}_r$ and $\hat{H}_{r2}$ indicate in turn the presence of additive variation, dominance variation and gene asymmetry. In the six-line diallel parent HY contains most of the commoner alleles and the greatest number of dominant alleles: OHO4 contains most recessive alleles and 38/11 most of the rarer alleles. The correlations between $\hat{p}_r$, $\hat{F}_r$ and $\hat{H}_{r2}$ describe (2.4) in general terms the properties of the gene distributions in the parents. Neither $\mathrm{corr}(\hat{p}_r, \hat{H}_{r2})$ nor $\mathrm{corr}(\hat{F}_r, \hat{H}_{r2})$, both with four degrees of freedom, is significant so that there is no suggestion that the asymmetry of the gene distributions favours one sign of allele or one sign of dominance. Only $\mathrm{corr}(\hat{p}_r, \hat{F}_r)=0.96$ is significant and this indicates that dominance is unidirectional towards larger yield. Evidently the negligible $\hat{F}$ is caused by a balance of asymmetry and not of dominance.

6.6. Graphs. Figures 1 and 2 contain graphs of W_{r_1} against W_{r_2} and of V_{r_1} against V_{r_2}, the former for the complete maize data and the latter for the six-line diallel. The line designations have been replaced by numbers as in Table 6. Also included are the best fitting points on the simple model (and the line of slope ½ through them) and on the model permitting gene correlation or parental heterozygosity. Both graphs have been drawn to the same scale of standard error and the smaller deviations from the lines of slope ½ in the second and epistasis-free graph are obvious.

It is clear that disturbances due to gene correlation and heterozygosity are not important in these data. The Figure 2 *W* graph indicates that multiple allelism is not important either, at least in the smaller diallel. As mentioned in 6.4 these factors may be important in other cases.

6.7. Experimental design. The numbers (four) of blocks used in these maize and cotton experiments are suited to our purpose. The recommended numbers of duplicates of each family in each block to suit our analysis are derived from the formulae of 5.3 and tabulated, to the nearest whole number, in Table 9. If reciprocal crosses are grown, then each reciprocal need have only half this number of duplicates. The total number of plots in each block is also included.

6.8. We can summarize our findings about the genetic control of maize yield in these ten inbred lines as follows. Epistasis is present and inflates F_1 yields. The epistatic F_1 yields are above the nonepistatic F_1 yields but the yields of the respective parents of the epistatic and nonepistatic F_1 families bear the same relation so that the dominance deviations of the two sets of F_1 families are about

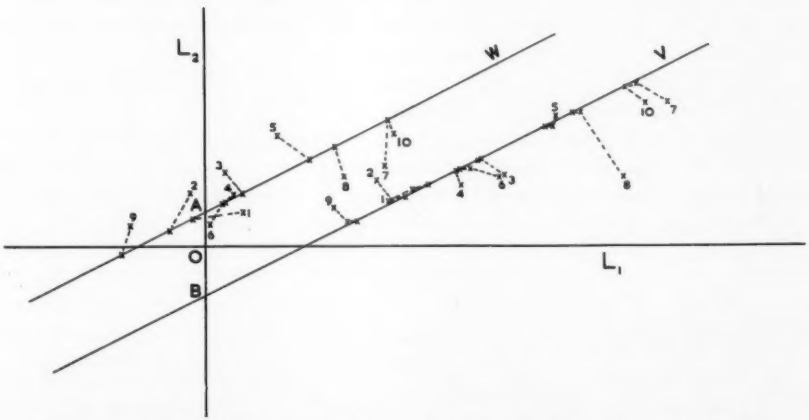

FIGURE 1.—The graph of W_{r_1} against W_{r_2} and of V_{r_1} against V_{r_2} for maize yield. Observed points are numbered: they are joined by broken lines to the best fitting points on a model allowing gene correlation and/or parental heterozygosity: these in turn are joined to the points which best fit the simple genetic model and which therefore lie on lines of slope ½. The latter two sets of points coincide in the W-graph.

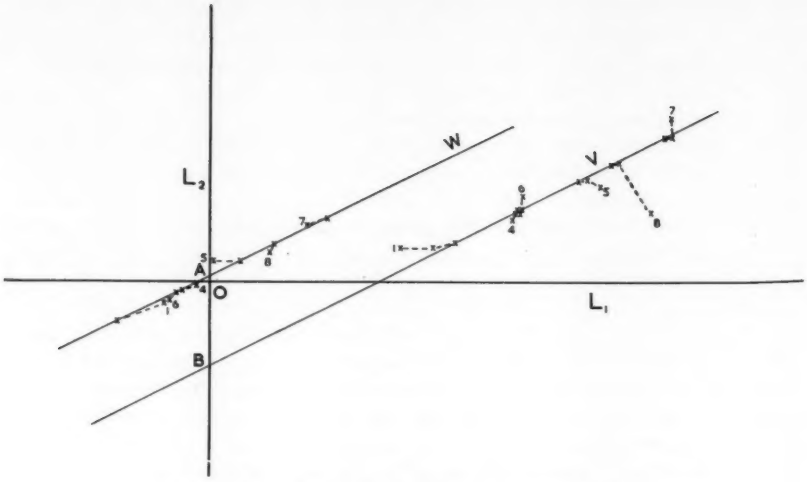

FIGURE 2.—As figure 1 for the epistasis-free maize diallel.

the same. A nonepistatic six-line subdiallel can be found and this happens to show more dominance or heterosis on the average than the complete data. The genetic system in these six lines is characterized by marked apparent overdominance for high yield though whether this is due to single genes or groups of genes we cannot say. The distributions of genes in the parents are not symmetrical and this asymmetry favours neither high nor low yield.

The cotton yields have not been investigated here to the same extent. HAYMAN (1957) discusses their epistatic properties.

The stability of family means is uniform within a generation in both maize and cotton but changes markedly from one generation to the next. The direction of change from inbred parents to F_1 families is unexplained and seems to fit no current theory.

TABLE 9

Recommended number of duplicates of each family for two levels of accuracy

		Maize		Cotton	
		p = .05	p = .01	p = .05	p = .01
Number of	Parent	2	2	2	2
duplicates	F_1	3	10	2	4
per family	F_2	2	5	2	5
Number of plots / block		245	695	72	142

7. SUMMARY

The diallel analysis of HAYMAN (1954b) is extended to include F_2 families. This greatly increases the accuracy of measurement of the components of genetic variation and of description of the gene distributions in the parents. The original diallel analysis was restricted to a certain simple genetic situation. The addition of F_2 data makes it possible, with the aid of HAYMAN's (1957) test for epistasis, to distinguish some of the more complicated genetic systems that may arise but it is still not possible to estimate the magnitude of the departure of any of these other systems from the simple one. Experimental designs to suit the diallel analysis are given.

The investigations of maize yield and cotton yield both reveal epistasis of the complementary type, in the former in high yielding crosses, and in the latter in low yielding crosses. In the six maize lines considered in more detail there is apparent overdominance for high yield; the line containing the greatest number of dominant alleles is determined. The gene distributions in these six lines are not symmetrical and the line containing the most of the commoner alleles is also determined.

LITERATURE CITED

ALLARD, R. W., 1956 The analysis of genetic environmental interactions by means of diallel crosses. Genetics **41**: 305–318.

DICKINSON, A. G., and J. L. JINKS, 1956 A generalized analysis of diallel crosses. Genetics **41**: 65–78.

HAYMAN, B. I., 1954a The analysis of variance of diallel tables. Biometrics **10**: 235–244.

1954b The theory and analysis of diallel crosses. Genetics, **39**: 789–809.

1957 Interaction, heterosis and diallel crosses. Genetics **42**: 336–355.

HULL, E. H., 1945 Regression analyses of yield of hybrid corn and inbred parent lines. Maize Genetics News Letter **19**: 21–27.

JINKS, J. L., 1954 The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. Genetics **39**: 767–788.

1956 The F_2 and backcross generations from a set of diallel crosses. Heredity **10**: 1–30.

KEMPTHORNE, O., 1956 The theory of the diallel cross. Genetics **41**: 451–459.

KINMAN, M. L., and G. F. SPRAGUE, 1945 Relation between number of parental lines and theoretical performance of synthetic varieties of corn. J. Am. Soc. Agron. **37**: 341–351.

LERNER, I. M., 1954 *Genetic Homeostasis*. Oliver and Boyd. Edinburgh.

MATHER, K., 1949 *Biometrical Genetics*. Methuen & Co. Ltd. London.

NELDER, J. A., 1953 Statistical models in biometrical genetics. Heredity **7**: 111–119.

TURNER, J. H., 1953 A study of heterosis in upland cotton. I. Yields of hybrids compared with varieties. II. Combining ability and inbreeding effects. Agron. J. **45**: 484–490.

WRIGHT, S., 1923 Mendelian analysis of pure breeds of livestock. I. The measurement of inbreeding and relationship. J. Heredity **14**: 339–348.

1952 The genetics of quantitative variability. *Quantitative Inheritance*, 5–41. H.M.S.O. London.

ON THE STABILIZATION OF A RING CHROMOSOME IN MAIZE

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A comparison of ring chromosomes in maize and Drosophila shows a striking dissimilarity in their behavior. Whereas all the rings described to date in maize are unstable, owing to the formation of dicentrics, the Drosophila rings are by comparison extremely stable. Recent studies on the unstable w^{vc} chromosome in Drosophila have been interpreted as indicating that the observed instability of this chromosome is independent of the ring structure (HINTON 1957). In contrast to Drosophila, two unstable ring chromosomes showing a high frequency of discentric double-sized rings at anaphase have been reported in human tumor tissue (LEVAN 1956). Studies of ring chromosome behavior are of importance in cytogenetic research because of their application to such problems as chromosome replication and recombination. This paper describes a stable ring chromosome in maize.

A chromosome 9 ring with the dominant markers *C* and *Wx* was produced by X-irradiation of pollen carrying a normal chromosome complement (Figure 1). This ring showed the typical unstable behavior giving rise to cytologically observed dicentric rings at anaphase, and in the proper genotype to kernels variegated for both *C-c* and *Wx-wx*. Two smaller rings were isolated from it, one carrying *C* but not *Wx*, and the other *Wx* but not *C*. Both of these were unstable. Variegated kernels carrying the *C* ring with the recessive *c* allele on the two normal homologues were planted and crossed to *c* pollen. All the resultant ears except one gave the expected colorless and *C-c* variegated kernels. The odd ear gave the low transmission of colored kernels expected with a trisomic fragment (16 percent); however, all these were uniformly colored. The absence of variegation was indicative of a change in the ring chromosome resulting in a stable fragment. This stability has been maintained through four generations of backcrossing.

Without an appreciable change in size, the stabilization of a ring chromosome could result as a consequence of one of two events: the opening out of the ring to form a rod chromosome fragment in which sister-strand exchanges do not give rise to dicentrics, or the maintenance of the ring structure with a modification in its behavior such that dicentric chromosomes are not formed, as appears to be the case with the rings in Drosophila. Although the small size of the ring makes the cytological studies difficult, the configurations observed favor the latter.

The stable ring is of about the same size as the small unstable ring from which it arose (Figure 2). At meiosis, the ring is most frequently collapsed upon itself,

[1] Operated by Union Carbide Nuclear Company for the U. S. Atomic Energy Commission.

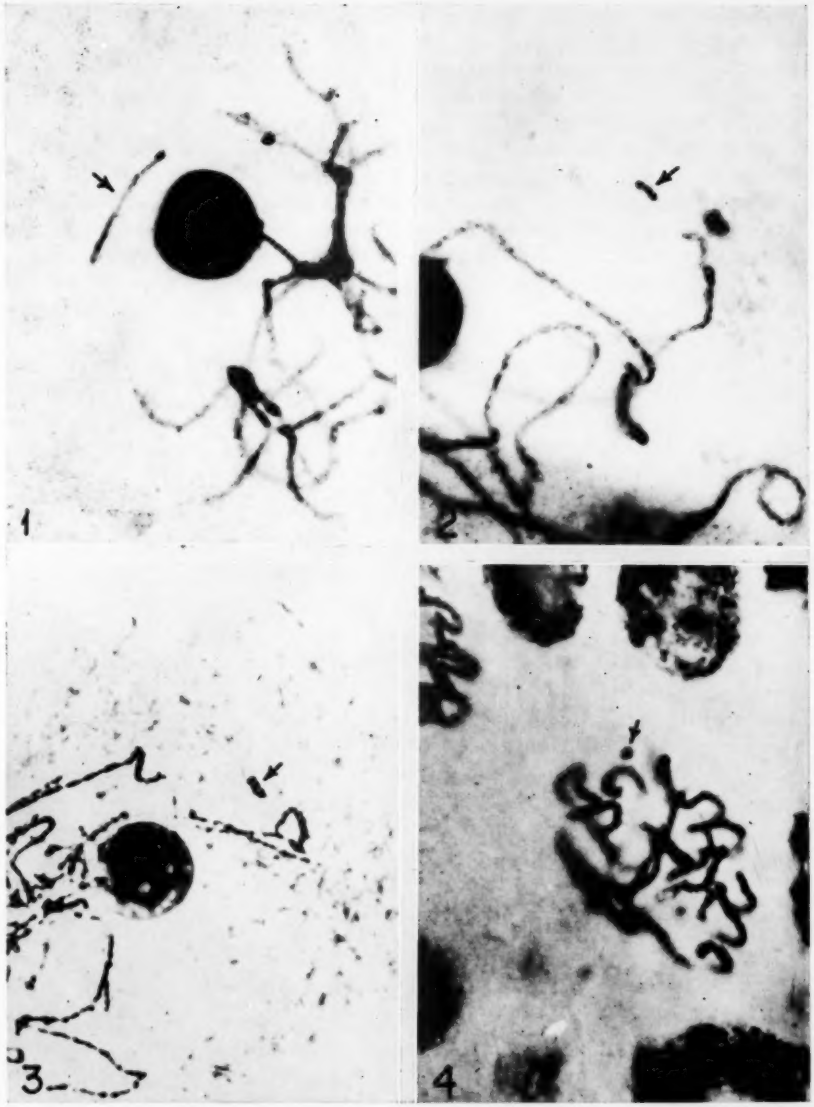

FIGURE 1.—Original unstable large ring—Pachytene.
FIGURE 2.—Small unstable *C* ring—Pachytene.
FIGURE 3.—Stable *C* ring—Pachytene.
FIGURE 4.—Stable *C* ring—root tip prophase.

but occasionally open ring configurations are observed (Figure 3). The ring-like structure is clearly seen in root tip mitotic prophase (Figure 4). Anaphases of root tip and glume mitoses and of the meiotic divisions were examined for dicentric double-sized rings. None were found. In all cases the rings were seen to disjoin freely from each other.

The differential behavior of Drosophila and maize ring chromosomes as related to instability is very perplexing. The possibility that this is a reflection of different mechanisms of chromosome replication in plants and animals is inconsistent with LEVAN's observations of high ring instability in man. The case against this is made even stronger by the finding of the two ring types in maize. Experiments have been conducted indicating that in maize ring behavior is dependent on the structure of the chromosome itself and not on the genotype or cytoplasm. Reciprocal crosses were made between plants homozygous for *c* and *wx* on the normal chromosomes carrying the stable *C* ring, and similarly marked plants carrying instead the unstable *Wx* ring. Both these rings are products of the larger ring, which carried both genes. By the use of these markers, those kernels that contained both the stable and unstable rings (the starchy colored kernels) could be recognized. If ring stability were under the genetic control of the nucleus or the cytoplasm, both rings would be expected to show identical behavior when present in the same cells. This was not the case. These kernels were nonvariegated for *C* and variegated for *Wx*. It seems clear therefore that ring stability is dependent on the structure of the ring chromosome itself.

The variegation studies are based on the classification of the phenotype, whereas the ring is recognized cytologically. To determine whether the color factor being studied is actually carried by the ring chromosome, root tips from colored and colorless sib kernels were sectioned and scored for the presence or absence of the ring chromosome in metaphase. Eighteen kernels of each class were scored. All the colorless were without the ring. Of the colored class, 15 showed the ring. The other three probably represent cases where the ring was present in the endosperm but lost to the embryo.

Occasional small colorless areas are found on the colored seeds, probably a result of nondisjunction of the ring in the endosperm mitoses. These can clearly be distinguished from variegation associated with ring instability by their appearance. Reversion to the variegated condition does occur however with a low frequency. From the crosses of *c/c* plus *C* ring × *c/c*, 1.4 percent of the colored class were variegated. There is about a 3-fold difference in the frequency of reversions to variegation depending on whether the ring was introduced through the male or the female in the backcross, the male being higher (Table 1). All of the variegated kernels from the male reversions were mosaic for colored and colorless tissue. Approximately two thirds of the female reversions were of this type while the remainder were variegated for depth of coloration, without any completely colorless areas on the kernels. The triploid endosperm receives two nuclei from the female and one from the male. In the latter class the endosperms received both a stable and unstable ring indicating that the reversions must have occurred

TABLE 1

Frequency of reversion to variegation in endosperms from reciprocal crosses

Cross	Colorless	Colored		Percentage colored	Percentage variegation in colored class
		Variegated	Nonvariegated		
c/c/C ring × *c/c* ♂	13,440	26	3117	18.9	0.8
c/c × *c/c/C* ring ♂	11,958	39	1410	10.8	2.7
Total	25,398	65	4527	...	1.4

in the female gametophyte. The colored-colorless variegated kernels can result from meiotic or premeiotic reversions or from a reversion in the first division of the gametophyte with an associated loss of the stable ring from the sister nucleus. That the reversions are post meiotic in origin is indicated by the lack of correlation between the endosperm and embryo in the variegated kernels. Difficulty in the detection of the variegation in those endosperms which had received both a stable and unstable ring could account for the low frequency of female reversions.

Eleven variegated kernels were available for crossing in an attempt to determine if the variegation involved a change in the ring back to the unstable condition. Two of the variegated kernels received the color factor from the male and the other nine from the female parent. For both variegated kernels from the male, the embryos carried the color factor but in the stable condition. These plants were used as female parents in backcrosses to tester plants, and showed the low transmission of the color factor. One plant gave 108 *c* : 35 *C*, and the other 129 *c* : 28 *C* : 2 *C-c* variegated. In the female, there is a definite correlation between variegation in the endosperm and loss of the sister ring chromatid to the embryo. Of the nine variegated kernels from the female, one showed the color factor in the embryo as well, with low transmission (129 *c* : 23 *C*). All the colored seeds were nonvariegated. Seven gave only colorless progeny, indicating the absence of the color gene in the embryo. Because of the dissimilarity between the endosperm and embryo in these ten cases it was not possible to determine the cause of the reversion to variegation.

In the case of the remaining variegated kernel it was possible to analyze the cause of the variegation. The *C* gene was transferred to one of the homologous rod chromosomes probably as a result of a crossover between the ring and the rod to form a dicentric chromosome with the *C* allele in the region between the two centromeres. The continuation of the fusion-bridge-breakage cycle would result in a variegated pattern in the endosperm (McCLINTOCK 1941). Healing of the broken end in the embryo would result in the transfer of the *C* marker from the ring to a stable rod chromosome as has been described previously for another ring in maize (SCHWARTZ 1953a). Pachytene configurations showing the chromosome with a terminal deficiency have been observed in the progeny. In addition to the *C* gene on the rod chromosome, the embryo received the noncrossover ring chromatid with its *C* gene so that the colored progeny are of three classes: (1) rod alone, recognized by 50 percent *C* kernels in the backcross progeny, (2) ring

alone giving a reduced transmission of *C*, and (3) both rod and ring, giving more than 50 percent *C*. Class 1 shows linkage between *C* and *Wx* as expected, class 2 independence, and class 3 an odd distribution resulting from the transfer of *c wx* and *c Wx* kernels into the phenotypic *C wx* and *C Wx* classes respectively because of the presence of the *C* ring (Table 2). There is some indication from the data of a non random recovery of the ring among the crossover and non-crossover classes; however, this is still under investigation.

TABLE 2

Phenotypic ratios of backcrosses to c wx ♂

Genotype of female parent	No. of ears	Phenotypic classes							
		C Wx		*C wx*		*c Wx*		*c wx*	
		No.	%	No.	%	No.	%	No.	%
C Wx/c wx	29	3141	40.7	748	9.7	777	10.1	3050	39.5
c Wx/*c wx*/*C* ring	3	30	3.9	33	4.3	376	49.2	325	42.5
C Wx/*c wx*/*C* ring	8	921	48.2	259	13.6	42	2.2	688	36.0

A single case of reversion to an unstable ring in the embryo was found. The associated endosperm was self-colored, not variegated, again indicating a postmeiotic origin for the reversion. In a backcross the colored progeny of this plant were all variegated (195 *c* : 40 *C*-*c* variegated). The ring was observed in both root tip prophases and pachynema and showed no detectable morphological differences from the parental stable ring from which it was derived. Double bridges resulting from dicentric rings have been observed in root tip anaphases. To date no premeiotic reversions giving instability in both the endosperm and embryo have been found, but many more variegated kernels are presently being tested. Instability in the embryo is detected by endosperm variegation in the next generation progeny.

Crossing over between sister chromatids has been proposed as the mechanism responsible for the formation of dicentric double sized rings (McCLINTOCK 1938; SCHWARTZ 1953b). This has gained support from the autoradiographic studies of TAYLOR *et al.* (1957) on the incorporation of tritium-labeled thymidine into the somatic chromosomes of *Vicia faba*. For the stable rings, one would have to postulate that either there are no sister strand crossovers or an even number of such exchanges always occurs. If the latter is true they would have to be such as to allow the rings to fall free without interlocking. It has been pointed out that the results of crossover studies with the stable ring chromosomes in Drosophila are equally consistent with no or an even number of sister strand exchanges (NOVITSKI 1955; SANDLER 1957). It is felt that the hope for distinguishing between these two possibilities lies in the tritium-labeling experiments; however the stable ring reported here is too small to make such experiments feasible.

SUMMARY

The behavior of a stable ring chromosome derived from an unstable ring is described. It is recognized as such by the absence of dicentric double sized rings at anaphase and of variegation for the markers that it carries. The stability is dependent on the structure of the ring itself. Unstable and stable ring chromosomes retain their individuality even when both are present in the same cells. Apparent postmeiotic reversions to the unstable condition are recovered.

LITERATURE CITED

HINTON, C., 1957 The analysis of rod derivatives of an unstable ring chromosome of *Drosophila melanogaster*. Genetics **42**: 55–65.

LEVAN, A., 1956 Self-perpetuating ring chromosomes in two human tumors. Hereditas **42**: 366–371.

MCCLINTOCK, B., 1938 The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376.

1941 The stability of broken ends of chromosomes in *Zea mays*. Genetics **26**: 234–282.

NOVITSKI, E., 1955 Genetic measures of centromere activity in *Drosophila melanogaster*. J. Cellular Comp. Physiol. Suppl. 2. **45**: 151–169.

SANDLER, L., 1957 The meiotic behavior of reversed compound ring X chromosomes in *Drosophila melanogaster*. Genetics **42**: 764–782.

SCHWARTZ, D., 1953a The behavior of an X-ray-induced ring chromosome in maize. Am. Naturalist **87**: 19–28.

1953b Evidence for sister-strand crossing over in maize. Genetics **38**: 251–260.

TAYLOR, J. HERBERT, PHILIP S. WOOD, and WALTER L. HUGHES, 1957 The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Natl. Acad. Sci. U. S. **43**: 122–128.

THE PRODUCTION OF TRANSLOCATIONS IN DROSOPHILA OOCYTES[1]

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THE availability of a great variety of stocks of *Drosophila melanogaster* has made it possible to carry out many experiments of elegant design. However, the study of chromosome breakage and rejoining in this organism has been limited by the failure of many breaks to rejoin in mature sperm until after sperm entrance. Furthermore, while breaks may rejoin rapidly in earlier stages of spermiogenesis (OSTER 1955) there is difficulty in making exact identification of stages involved in irradiation, and obtaining replicable samples of sperm is correspondingly difficult.

The finding (PARKER 1953) that radiation-induced detachment of attached-X chromosomes in Drosophila is a translocation process has given another method for the study of breakage and rejoining in this organism. To recover viable detachments, the attached-X must be broken in the proximal heterochromatin. PARKER and MCCRONE (1958) believe that the recovered detached X is usually the centric X, the broken end of which has rejoined with the acentric tip of another broken chromosome. The reciprocal product is occasionally recovered, the acentric X combining with the centric fragment of another chromosome. The recovery of a detachment depends upon subsequent disjunction of the two X's involved in the rearrangement. For this reason, this type of process has been called, perhaps unfortunately, "half-translocation," which implies failure of the other half of the translocation to be generated (HERSKOWITZ and ABRAHAMSON 1956; ABRAHAMSON, HERSKOWITZ and MULLER 1956). Since these exchanges in all likelihood are chromatid translocations (PARKER and MCCRONE 1958), non randomness of second division segregation together with the possibility of separation at the first division make it quite unlikely that both products might be included in the egg nucleus. Because of the way these translocations are recovered, we know only that the other chromosome fragments either have been lost or have been included in polar nuclei. The writers prefer simply to use the term translocation to describe the processes involved.

Analysis of a number of the detachments obtained has shown that in most cases the new end of the broken X may be identified. PARKER (1954a,b) found that if a Y chromosome be present, it most often supplies the other end for the detached X; in the absence of a Y, translocations with chromosome 4 are most

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frequent. There is, somewhat infrequently, involvement of breaks near the tips of the major autosomes, and occasionally deletion of nearly an entire X (HERSKOWITZ and MULLER 1953; ABRAHAMSON, HERSKOWITZ and MULLER 1956).

SONNENBLICK (1950) states that meiosis is arrested, and does not proceed beyond Metaphase I until sperm entrance. From this, it may be inferred that all breaks rejoin prior to the completion of meiosis, otherwise the acentric fragments would not be expected to be included in the egg nucleus, and two-break events would not be recoverable. This should allow measurement of the time required for breaks to rejoin, using variation in dose rate, or fractionation of the treatment as has been done with Tradescantia and other organisms (SAX 1939; and others).

The sensitivity of oocytes to ionizing radiations was found by PATTERSON, BREWSTER and WINCHESTER (1932) to increase with age. This has been confirmed by PARKER (1955) for detachment of attached-X and for dominant lethals, and by KING, DARROW and KAYE (1957) for recessive and dominant lethals. KING, RUBINSON and SMITH (1956) have described oogenesis in adult Drosophila and have designated 14 stages. It seems clear from their description that the cells being treated in the newly emerged females are in stage 7 and those in females three days old or older are in stage 14.

This change in sensitivity with age raises a number of questions relevant to the translocation processes. The experiments here reported are attempts to see if dose response is "two hit" in oocytes of various ages, how long breaks remain open, and if the increase in sensitivity of the oocyte is associated with any change in the rate of rejoining of breaks. PARKER and HAMMOND (1957) have reported these results in abstract form.

EXPERIMENTAL METHODS

In preliminary experiments, cited in Table 1, attached-X chromosomes marked with *br ec* or with *y* were used; in all other experiments an attached-X marked with y^2 *su-*w^a w^a *bb* was used. The attached-X females also carried a modified Y, sc^9·Y. This is a "complete" Y chromosome in that it supplies all Y chromosome requirements for male fertility; in addition it carries the normal alleles of yellow and achaete, allowing ready identification of a number of translocations by linkage of yellow$^+$ with the detached X.

Virgin attached-X females, raised at a temperature of 23.5-24°C, and differing in age by no more than 12 hours were irradiated either shortly after emergence, or were aged from three to six days at a temperature of 25°C before being irradiated. In the experiments given in Table 1, these females were mated individually with Muller-5 males immediately after irradiation, being removed three days later. In the other experiments, mass matings of 25 females with 25 Muller-5; *ci* ey^R males in half pint culture bottles were used; young females were aged two days after irradiation before mating. In view of the great differences in sensitivities of oocytes of stages 7 and 14, females were allowed to lay eggs for no more than 24 hours before being removed. Under these conditions, an average of about 20 eggs per female will be laid.

TABLE 1

Frequency of detachment in young females (stage 7 oocytes)

Females irradiated	Dose, r	*br ec*, or *y* ♀♀	w^a *B* ♂♂	*B* ♀♀	*br ec*, or *y* ♂♂	Percentage detachment
br ec/Y	250	2300	2461	1	1	0.04± .04
br ec/Y	500	1190	1309	3	1	0.16± .08
br ec/Y	1000	746	788	7	9	1.03± .26
y/Y	250	2133	1306	1	2	0.09± .05
y/Y	500	1444	974	5	2	0.29± .11
y/Y	1000	586	316	6	7	1.42± .40

The irradiations referred to in Table 1 were made using an air-cooled Coolidge tube operated at 55 KVP with 1 mm of Aluminum filtration. Treatments indicated in Table 2 were given using similar equipment operated at 60 KVP. The dose rates were 100r/minute in each of these. Later work was carried out using a G. E. Maximar-100 machine operated at 100 KVP, with 1 mm of Aluminum filtration, giving a HVL of 1.3 mm of Aluminum. The dose rate was approximately 290r/minute at a target distance of 15 cm. All dosimetry was with a Victoreen r-meter with a 100-r thimble chamber; in all work except that shown in Table 1, the instrument used had been calibrated against a Cobalt-60 source in the Biology Division of Oak Ridge National Laboratory.

Unetherized flies were placed in acrylic plastic containers during irradiation. In the case of interrupted treatments, if the time interval between irradiations exceeded five minutes, flies were returned to culture vials without etherization. All irradiations were carried out at temperatures between 23° and 26°C.

All frequencies were calculated on the basis of total flies counted, and are crude percentages differing in this respect from the method previously used (PARKER 1954a,b). In previous publications, due to the variation in viability of Muller-5 males depending on the type of Y chromosome carried, regular male classes were disregarded and the frequency determination was based on the number of regular females and detachments, with correction for the estimated numbers of inviable or poorly viable classes.

TABLE 2

Frequency of detachment in females aged five days before irradiation (stage 14 oocytes)

Dose, r	*su-w*^{a} w^a ♀♀	*y*$^+$ *su-w*^{a} w^a ♀♀	w^a *B* ♂♂	w^a *B* ♀♀	*y*$^+$ *su-w*^{a} w^a ♂♂	*su-w*^{a} w^a ♂♂	Percentage detachment
0	4	2878	2664	1	0	1	0.04± .03
100	6	2685	2684	9	0	1	0.19± .06
200	5	2185	2162	18	9	0	0.62± .12
250	1	2581	2654	31	8	3	0.80± .12
400	2	1214	1273	30	14	3	1.85± .27
500	3	1691	1864	58	27	3	2.41± .25
800	0	362	420	23	6	0	3.58± .65
1000	0	269	306	11	6	4	3.52± .75

RESULTS

Dose response in stage 7 oocytes

PARKER (1953) irradiated newly emerged females (aged 0–12 hours), and immediately mated them individually in culture vials, where they remained three days before being discarded. Two different attached-X lines were used, *br ec*/Y and *y*/Y. Table 1 gives the results of these irradiations. In the range of 250–1000r, the frequency of detachment seemed to vary approximately as the square of the dose. PARKER (1954a) found the increase with dose somewhat less in the range 1000–2000r.

Dose response in stage 14 oocytes

The conclusion of SONNENBLICK (1940) that dominant lethals induced in oocytes of females aged five days resulted from the failure of breaks to rejoin raised the possibility that no recoverable two-hit detachments could be produced in this age, or that dominant lethals were produced in some manner other than the one postulated. It has been found that dominant lethals in young oocytes are produced in some "two hit" manner, while in aged cells their origin is "one hit" (PARKER 1955; KING, DARROW and KAYE 1957). Obviously, the technique of detachment of attached-X's gives a method of answering the question of the ability of breaks induced in these older (stage 14) cells to rejoin.

The range of dosages used was from 100 to 1000r; as the fraction of eggs surviving is about $e^{-1.77D}$ (where D is measured in Kr) it is not feasible to use dosages above 1000r, and the recovery of offspring is very poor even at that dose. Table 2 gives the results obtained with females aged five days before treatment. A good "two hit" relationship is found at lower doses; it is interesting to see a possible levelling of the detachment frequency at higher doses. Possibly oocytes of two different sensitivities are included in the sample, with the more sensitive stage 14 which is more likely to yield detachments also being much more readily killed. The apparent "break" in the otherwise linear survival curve of SONNENBLICK (1940) perhaps indicates the presence of a few cells of much lower sensitivity (stage 7?) in his samples.

Dose-fractionation experiments with stage 7 oocytes

Attached-X females were given a total dose of 2000r, either in one uninterrupted dose, or in two fractions of 1000r each, with a measured interval between the two treatments. The results of two series of irradiations are given in Table 3. It will be seen that all treatments involving intervals of ten minutes or more give frequencies which are significantly lower than the continuous-irradiation controls. The values obtained vary around about 70 percent of the control frequency.

There is a marked difference between frequencies obtained in the first and in the second series of experiments, these being carried out about two months apart. A similar difference is to be seen in experiments in the aged series, carried out at about the same time (Table 4). Methods of treatment were as nearly similar as

TABLE 3

Effects of dose-fractionation in young females (stage 7 oocytes)
Total dose 2000r, divided into two 1000r fractions separated by measured time interval

	Time interval	*su-w*^{a} *w*^{a} ♀♀	*y*^{2} *su-w*^{a} *w*^{a} ♀♀	*w*^{a} *B* ♂♂	*w*^{a} *B* ♀♀	*y*^{2} *su-w*^{a} *w*^{a} ♂♂	*su-w*^{a} *w*^{a} ♂♂	Percentage detachment	Difference	D/s.e.
First series	0	59	5053	4739	225	71	72	3.60± .18	—	—
	15 minutes	10	1149	1165	30	19	13	2.55± .32	−1.05± .37	2.84
	30 minutes	28	2964	2638	55	33	27	2.00± .20	−1.60± .27	5.93
	45 minutes	28	2443	2162	74	22	22	2.69± .23	−0.91± .29	3.14
	60 minutes	39	3043	2889	102	40	30	2.80± .21	−0.80± .28	2.86
Second series	0	34	5249	5021	144	65	53	2.48± .15	—	—
	5 minutes	31	4393	4230	114	46	38	2.24± .16	−0.24± .22	1.09
	10 minutes	41	5202	4875	109	46	28	1.78± .13	−0.70± .20	3.50*

* Difference 5-10 minutes is 0.46± .21, which is 2.2 times its standard error.

TABLE 4

Effects of dose-fractionation in aged females (stage 14 oocytes)
Total dose 500r, divided into two 250r fractions separated by measured time interval

	Time interval	*su-w*^{a} *w*^{a} ♀♀	*y*^{2} *su-w*^{a} *w*^{a} ♀♀	*w*^{a} *B* ♂♂	*w*^{a} *B* ♀♀	*y*^{2} *su-w*^{a} *w*^{a} ♂♂	*su-w*^{a} *w*^{a} ♂♂	Percentage detachment
First	0	4	1900	2286	95	31	8	3.10± .26
series	15 min.	3	1957	2467	72	28	7	2.36± .23
(5-day)*	30 min.	4	1682	1969	86	41	6	3.51± .30
Second	0	4	2592	2815	85	36	5	2.28± .20
series	1 hour	8	2539	2831	108	34	7	2.87± .22
(5-day)*	2 hours	12	2875	3147	111	43	12	2.68± .21
Third†	0 (5-day)*	7	2318	2392	89	17	9	2.38± .22
series	0 (6-day)*	2	2001	2149	65	12	5	1.94± .21
	24 hours	4	2393	2566	91	25	7	2.42± .22
Fourth‡	0 (3-day)*	15	2607	2672	87	32	5	2.28± .20
series	24 hours	5	1522	1627	51	17	2	2.17± .26

* Figures in parentheses give age at time of irradiation.
† In the third series, all females were mated when six days old.
‡ In the fourth series, all females were mated when four days old.

possible. There can be no clustering of identical translocations, since all cells were oocytes at the time of treatment. Furthermore, PARKER (1953) found that the detachments fell in a Poisson distribution in the progeny of individual treated females. Again, this difference may be the result of heterogeneity in the samples.

Dose-fractionation experiments with stage 14 oocytes

This series of experiments was carried out in essentially the same way as the preceding, except that the dosage chosen for these irradiations was 500r. As seen in Table 2, the recovery of offspring at doses of 800r or more is so low as to make it impractical to use these higher dosages. Also, the frequency obtained at 500r is roughly similar to that found at 2000r in stage 7 oocytes.

The results of these experiments are given in Table 4. With the exception of the fifteen minute interval group in the first series, in no case does the fractionated treatment differ significantly from the control, nor is there any suggestion of a lowering of the frequency as a result of the intermittent treatment. In the one case, the fifteen minute interval, the difference is greater than twice its standard error, $0.74 \pm .35$ percent. The finding of a higher frequency in the 30 minute group makes it likely that this difference is the result of some unknown experimental error, or of sampling errors, rather than representing a real reduction in translocation frequency.

The results are quite uniform for females treated at ages of from three to six days except that, as in Table 3, the first series is somewhat higher than the later ones. It seems clear that after three days of aging there is no further increase in sensitivity of oocytes.

DISCUSSION

HERSKOWITZ and ABRAHAMSON (1956) have irradiated females aged three or more days, and from various findings have concluded that the production of detachments in their material is dose-rate dependent, and that the breaks induced in this material rejoin fairly rapidly. There are certain difficulties brought about by their experimental procedures which might cause one to question the plausibility of the conclusions reached. By allowing females to oviposit over a period of several days, they have introduced a great deal of heterogeneity of sensitivity, as is shown in the results of this paper. KING, RUBINSON and SMITH (1956) state that in actively ovipositing females, there will be stage 14 oocytes in the most posterior follicle of the ovariole, followed immediately by a follicle containing a stage 7 oocyte. The differences in survival of stages 7 and 14 at 2000r (the dose most used by HERSKOWITZ and ABRAHAMSON) would allow one to recover some 80 percent of stage 7 cells, while only three percent of the more sensitive stage 14 cells would survive. It would seem that most of the F_1 flies recovered in the Indiana experiments must therefore have developed from oocytes of much less sensitivity than those dealt with in the aged females in the present paper. With this modification, their results do not differ greatly from the ones here reported.

The results obtained in the fractionation experiments allow the conclusion that breaks do rejoin rapidly, in about ten minutes in stage 7 oocytes but remain open for a long period of time in stage 14. A comparison of the second and third series in Table 4 shows that there is no loss of detached X's resulting from an additional day of aging after irradiation. In the second series, by the time the female is six days old (24 hours after irradiation) eggs in the sample have all been laid, hence all the rejoining which will be possible has been completed. In the third series, a comparison of the fractionated series with those given uninterrupted irradiation shows that at the time the flies become six days old, breaks induced 24 hours earlier are still open and able to interact with those induced by the second treatment. In one case, breaks have rejoined within 24 hours, while in the other these breaks have all remained open for 24 hours. The conclusion seems inescapable that breaks remain open until about the time the egg is laid; one would suspect sperm entrance as being the event which triggers rejoining, as is the case with the rejoining of breaks induced in sperm (MULLER 1940).

The discovery by WOLFF and LUIPPOLD (1955) of the role of oxidative metabolism and ATP formation in the rejoining of radiation-induced breaks in chromosomes suggests that perhaps the difference in the behavior of the two stages dealt with here, i.e., failure of breaks to rejoin in older cells, may be due to changes in oxidative metabolism during meiotic prophase. Such changes in respiratory pattern of eggs during maturation and fertilization have been reviewed by BRACHET (1950). In the sea urchin, for example, there is a decrease of oxygen consumption during maturation of the egg; the unfertilized egg's respiration is not inhibited by carbon monoxide nor by cyanide, while in the fertilized egg respiration is inhibited by these poisons. Apparently, the amount of cytochrome oxidase does not

change with fertilization, but an intermediary transport compound is formed at that time. The existence of a similar situation in the Drosophila oocyte would account for the differences in rejoining rates in the two stages.

STONE, HAAS, ALEXANDER and CLAYTON (1954) and HAAS, DUDGEON, CLAYTON and STONE (1954) have shown an increased production of translocations in an atmosphere containing carbon monoxide. STONE (1956) gives evidence from the difference in response of poky and of wild type Neurospora irradiated in an atmosphere containing carbon monoxide that cytochrome oxidase protects against radiation damage. It may be permissible to speculate that the changes in sensitivity of Drosophila oocytes may in part be due to changes in the activity of cytochrome oxidase, which is also reflected in ability of breaks to rejoin. Experiments are under way to test this hypothesis, using dominant lethals as a measure of radiation damage.

Another striking difference between the young and aged series is the relative difference in sizes of the three detachment classes recovered. In the young series, nearly as many males as females are obtained, and the males are about equally divided between those carrying yellow$^+$ and those showing yellow^2. In the aged series, there is a marked deficit of males as compared to females, and only about a fifth of the males carry the marker, yellow$^+$, from the sc^8-Y. PARKER and MCCRONE (1958) show these differences to be due to a relative increase in autosomal translocations and gross X deficiencies over X-Y translocations. The possible relation of this finding to radiation sensitivities of cells is still obscure, in that it might result from changes in proximity and coiling of the chromosomes, or from the delayed rejoining of the broken ends.

SUMMARY

Attached-X females of various ages were irradiated, and the frequency of detachment determined in females of various ages. There is an increase in radiation sensitivity of oocytes with aging up to three days, after which there is no evident increase. A "two hit" formation of detachments is found both in young (stage 7) and old (stage 14) oocytes. Dose fractionation experiments show breaks in stage 7 rejoin in about ten minutes, while those in stage 14 do not rejoin until fertilization. There is evidently a change in the proportions of the various kinds of exchanges obtained when females are aged before treatment. Speculations are made as to the possible role of cytochrome oxidase activity in the changing sensitivity of oocytes.

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LITERATURE CITED

ABRAHAMSON, S., I. H. HERSKOWITZ, and H. J. MULLER, 1956 Identification of half-translocations produced by X-rays in detaching attached-X chromosomes of *Drosophila melanogaster*. Genetics **41**: 410–419.

BRACHET, J., 1950 *Chemical Embryology*. Interscience Publishers, Inc. New York.

HAAS, F. L., E. DUDGEON, F. E. CLAYTON, and W. S. STONE, 1954 Measurement and control of some direct and indirect effects of X-radiation. Genetics **39**: 453–471.

HERSKOWITZ, I. H. and S. ABRAHAMSON, 1956 Induced changes in female germ cells of Drosophila. I. Dependence of half-translocation frequency upon X-ray delivery rate. Genetics **41**: 420–428.

HERSKOWITZ, I. H., and H. J. MULLER, 1953 Evidence against healing of X-ray breakages in chromosomes of female *Drosophila melanogaster*. Genetics **38**: 669. (Abstr.).

KING, R. C., J. B. DARROW, and N. W. KAYE, 1956 Studies on different classes of mutation induced by radiations of *Drosophila melanogaster* females. Genetics **41**: 890–900.

KING, R. C., A. C. RUBINSON, and R. F. SMITH, 1956 Oogenesis in adult *Drosophila melanogaster*. Growth **20**: 121–157.

MULLER, H. J., 1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40**: 1–66.

OSTER, I. I., 1955 Modification of X-ray mutagenesis in Drosophila. I. Reunion of chromosomes irradiated during spermiogenesis. Genetics **40**: 692–696.

PARKER, D. R., 1953 Observations on X-ray induced detachments of attached-X chromosomes in Drosophila. J. Tenn. Acad. Sci. **28**: 185. (Abstr.).

1954a Radiation induced exchanges in Drosophila females. Proc. Natl. Acad. Sci. U. S. **40**: 795–800.

1954b Role of the Y chromosome in induced detachment of attached-X chromosomes in *Drosophila melanogaster*. Genetics **39**: 986. (Abstr.).

1955 The origin of dominant lethals in irradiated oocytes of Drosophila. Genetics **40**: 589. (Abstr.).

PARKER, D. R., and A. E. HAMMOND, 1957 Chromosome breakage and rejoining in oocytes of *Drosophila melanogaster*. A. S. B. Bulletin **4**: 14. (Abstr.).

PARKER, D. R., and J. MCCRONE, 1958 A genetic analysis of some rearrangements induced in oocytes of Drosophila. Genetics **43**: (In press.)

PATTERSON, J. T., W. BREWSTER, and A. M. WINCHESTER, 1932 Effects produced by aging and X-raying eggs of *Drosophila melanogaster*. J. Heredity **23**: 325–333.

SAX, K., 1939 The time factor in X-ray production of chromosome aberrations. Proc. Natl. Acad. Sci. U. S. **25**: 225–233.

SONNENBLICK, B. P., 1940 Cytology and development of the embryos of X-rayed adult *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **26**: 373–381.

1950 The early embryology of *Drosophila melanogaster*. *Biology of Drosophila*. Ch. II: 62–167. John Wiley and Sons, Inc. New York.

STONE, W. S., 1956 Indirect effects of radiation on genetic material. Mutation. Brookhaven Symposia Biol. **8**: 171–190.

STONE, W. S., F. HAAS, M. L. ALEXANDER, and F. E. CLAYTON, 1954 Comments on the mechanism of action of radiations on living systems. Univ. Texas Publ. **5422**: 244–271.

WOLFF, S., and H. E. LUIPPOLD, 1955 Metabolism and chromosome break rejoining. Science **122**: 231–232.

COMPETITION BETWEEN WILD TYPE ISOALLELES IN EXPERIMENTAL POPULATIONS OF DROSOPHILA MELANOGASTER[1]

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GENETIC variability in Mendelian populations is indispensable for the operation of natural selection. Although on outward appearance, the members of a given species in nature are phenotypically similar, experimental methods reveal that a supply of concealed variability is usually present. This was first reported in Drosophila by CHETVERIKOV (1926) and subsequently confirmed for other organisms by numerous workers.

In Drosophila much of this genetic variation exists in the form of recessive mutant alleles present in heterozygous condition. In the homozygous state, such genes, whether or not they produce obvious morphological or physiological traits, usually depress the viability of their carriers. It is often tacitly assumed that the wild type allelic form at any one locus is unvarying. Nevertheless, demonstrations that genetic variation can occur among these so-called normal alleles have been made by TIMOFEEFF-RESSOVSKY (1932), MULLER (1935), SPENCER (1938, 1944), STERN and SCHAEFFER (1943), and LEFEVRE (1955). In each of these studies two or more wild type alleles were indistinguishable under normal circumstances. Special tests were required in order to differentiate them. STERN and SCHAEFFER proposed the term isoalleles to apply to alleles which behaved in this fashion.

Since the pioneer work of L'HERITIER and TEISSIER (1934, 1937), extensive experimentation has been carried out to determine the status of mutant genes in wild populations. Similar work involving differing normal alleles has not been previously undertaken. The present study is an attempt to elucidate the significance, at the population genetics level, of wild type isoalleles at the cubitus interruptus (*ci*) locus in *Drosophila melanogaster*. Of particular interest is the determination of the role which natural selection plays in populations containing two different wild type isoalleles.

Wild type isoalleles at the ci *locus*

Normal alleles at this fourth chromosome locus can be divided into two categories on the basis of their dominance relationships with the mutant allele *ci*. Whereas one group of these alleles is completely dominant over *ci*, the second type permits a certain number of ci^+/ci flies to express the mutant phenotype, characterized by interruptions of the fourth vein of the wing. The alleles com-

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prising this latter group vary among themselves in the proportion of ci^+/ci individuals which exhibit the mutant phenotype (STERN and SCHAEFFER 1943). All alleles, whether completely or only partially dominant over the *ci* allele, are indistinguishable from one another in the homozygous (ci^+/ci^+) condition, with some possible exceptions at very low temperatures. Observations of various ci^+ alleles in the hemizygous condition (through use of the *M-4* deficiency) is another method of differentiating certain of them. Examining these wild type alleles under other unusual conditions, whether genetic or environmental, may divulge additional means of separating them. However, under ordinary circumstances, such as exist in natural populations or laboratory cultures, they cannot be distinguished and hence are termed wild type isoalleles.

It would appear desirable to test for possible selective differences between alleles of this sort. Recent studies of variability in natural populations of Drosophila by DOBZHANSKY and SPASSKY (1953, 1954) and WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE (1953) suggest that instead of a single "normal" genotype in nature, there are numerous alleles at many loci. If isoalleles are a common phenomenon, do they serve merely to promote a high level of heterozygosity within a population? Are they as SLATIS (1955) states, ". . . by definition, of little importance to the vitality of the individual."? The investigation herein reported may help to answer these questions.

METHODS

The population cage technique was utilized for all selection experiments. Essentially similar to the one employed by WRIGHT and DOBZHANSKY (1946), the cage in question differs in three details. It is slightly larger, has 16 instead of 15 circular holes, and contains screens on three sides, as compared to the model with a single side screened used earlier. The population supported by this container approximates 4,000 flies.

At the inception of an experiment a given number of unmated females and males were etherized, placed in a dry empty Petri dish, and introduced into the cage. Two food dishes were installed at this time also. The food was a standard cornmeal-molasses-agar-yeast blend to which some live yeast suspension was added. Two days later two additional food dishes were placed in the cage and the empty dish removed. Thereafter, two food dishes were added every other day until the maximum of sixteen was reached. Two days following this, the two oldest dishes were replaced with fresh ones. This practice was continued until the termination of the experiment. When a food dish was discarded it usually contained only a small amount of dried medium and empty pupal cases. At times, however, the medium was still moist and contained living larvae which were therefore lost to the population. A few adults escaped occasionally during the replacement of dishes.

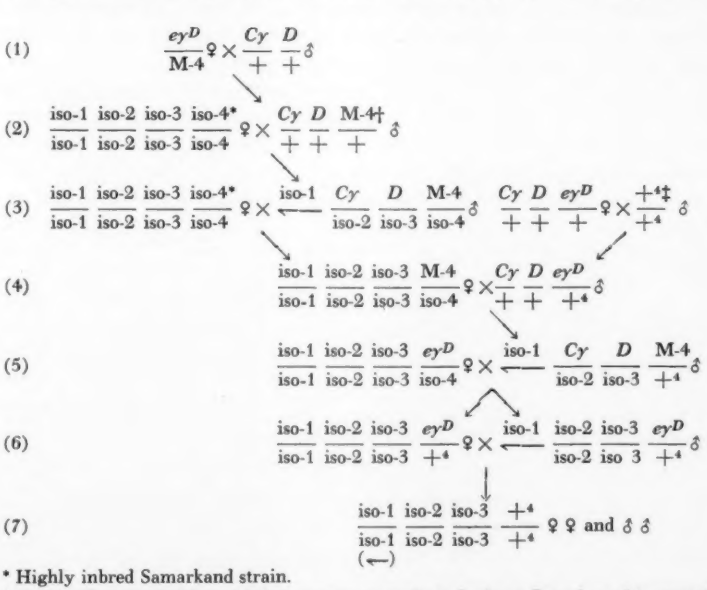
The experimental populations were kept at $25 \pm 1°C$. Replications placed at $17 \pm 1°C$ were lost before they could yield analyzable results.

In one set of experiments two wild type isoalleles at the *ci* locus were placed in

competition. The allele $+^4$, derived from an Iowa wild type stock, is completely dominant over the mutant allele *ci*. On the other hand, $+^3$, of uncertain laboratory origin (STERN and SCHAEFFER 1943), when heterozygous with the *ci* allele at 25°C, causes 77 to 100 percent of males to have interrupted fourth veins. Penetrance is somewhat lower among females. The three genotypes, $+^4/+^4$, $+^4/+^3$ and $+^3/+^3$ all confer a normal phenotype.

In an attempt to confine genetic variation to the fourth chromosome, two stocks were made isogenic for chromosomes I, II and III. These three chromosomes were derived from a wild type stock, iso-Samarkand, inbred by single pair brother-sister matings for over 200 generations. A diagram of this procedure is given in Figure 1. The resulting stocks differ in that one is homozygous for the fourth chromosome which carries $+^4$ while the other has in double dose the $+^3$-containing chromosome. The two stocks were designated isogenic $+^4$ and isogenic $+^3$, but in the interest of brevity, will hereafter be called simply $+^4$ and $+^3$.

Unmated flies of these two stocks comprised the original population in three cage experiments. In cage 1, initiated December 22, 1954, the starting population consisted of 100 $+^3$ males, 100 $+^3$ females, 100 $+^4$ males and 100 $+^4$ females. Thus, the original allele frequencies were $p(+^4)=0.5$, $q(+^3)=0.5$. Cage 3 was begun


(1)
(2)
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(5)
(6)
(7)
iso-1 iso-2 iso-3 iso-4* ♀ × Cy D M-4† ♂
iso-1 Cy D M-4 ♂
Cy D ey^D ♀ × +^4‡ ♂
iso-1 iso-2 iso-3 M-4 ♀ × Cy D ey^D ♂
iso-1 iso-2 iso-3 ey^D ♀ × iso-1 Cy D M-4 ♂
iso-1 iso-2 iso-3 ey^D ♀ × iso-1 iso-2 iso-3 ey^D ♂
iso-1 iso-2 iso-3 +^4 ♀ ♀ and ♂ ♂
(←)

* Highly inbred Samarkand strain.
† *Cy D ey^D* ♂ crossed in a similar manner to produce the iso *ey^D* stock used in step (5).
‡ $+^3/+^3$ and *ci ey^R*/*ci ey^R* ♂ ♂ crossed at this time also.

FIGURE 1.—Crossing scheme employed to produce $+^4$, $+^3$ and *ci ey^R* stocks homozygous for a fourth chromosome and isogenic for chromosomes I, II and III derived from the Samarkand wild type strain inbred by single pair, brother-sister matings for more than 200 generations.

in a similar fashion on December 30, 1954, although the starting allele frequencies were $p(+^4)=0.3$, $q(+^3)=0.7$. Conversely in cage 5, started January 12, 1955, the initial values were $p(+^4)=0.7$, $q(+^3)=0.3$.

To estimate the frequencies of the $+^4$ and $+^3$ alleles, samples of each cage population were taken at intervals of one month or longer. By aspiration, 120 adult males were removed from individual cages. Each male was etherized and crossed to two or three virgin females of the genotype *ci ey*^{R}/*ci ey*^{R}, in one ounce creamers at 25°C. Five to seven days later, the males were returned to their cage and the females discarded. About the fourteenth day, male progeny in the first 100 fertile creamers were classified. The two wings of each son were examined for the presence of interrupted fourth veins. Fifty sons were observed where possible, although smaller numbers (minimum of 30) were encountered in occasional creamers. The genotype of the male parent could be determined by this classification of his sons. If none of the sons exhibited vein interruptions, the father was classified $+^4/+^4$. When from 77 to 100 percent of the male progeny were *ci* in phenotype, the male parent was considered to be $+^3/+^3$. Finally, heterozygous $+^4/+^3$ fathers produced sons, 17 to 57 percent of which were *ci* in appearance. There was no overlap between these three distinct classes. In several instances, where the frequency of *ci* phenotypes was in the "high 50's" or "low 80's", additional tests were performed which, in all cases, substantiated the correctness of assigning the male parent on the basis outlined above.

All flies competing in cages 1, 3 and 5 were phenotypically normal. A second experimental series was instituted in which the recessive gene *ci* was involved. The original population of cage 7, initiated July 13, 1955, consisted of $+^3$ homozygotes as well as flies of the genotype *ci ey*^{R}/*ci ey*^{R}. The *ci ey*^{R} stock had previously been made isogenic for chromosomes I, II and III derived from the iso-Samarkand strain already described. Initial frequencies in cage 7 were $p(+^3)=0.3$, $q(ci\ ey^R)=0.7$. Actual numbers of flies were 30 $+^3$ females, 30 $+^3$ males, 70 *ci ey*^{R} females and 70 *ci ey*^{R} males. Cage 8 was similarly stocked on July 13, 1955 with $+^4$ and *ci ey*^{R} flies, with original frequencies of $p\ (+^4)=0.3$, $q(ci\ ey^R)=0.7$. The starting population of cage 9 on July 15, 1955 consisted of both wild type stocks as well as the double recessive *ci ey*^{R}. The initial frequencies were $p(+^4)=0.2$, $q(+^3)=0.2$ and $r(ci\ ey^R)=0.6$.

Certain changes in the sampling methods of these three cage populations were necessitated by the fact that the presence of the *ci ey*^{R} chromosome led to the occurrence of phenotypes other than wild type. A sample of 120 males was removed from a cage. Of this number, 100 were picked at random and classified into four classes; wild type, *ci* but normal for eye size, *ci* and eyeless, and finally eyeless only. If any of the remaining 20 males were normal in appearance they were progeny-tested in the manner indicated below. All creamers containing wild type males to be tested, whether belonging to the 100 actually classified or the additional 20, were kept together on a single tray. At the time when the F_1 flies were examined, fertile creamers were selected at random from the entire group, the number of creamers selected equalling the number of males classified pre-

viously as wild type. This procedure was followed to insure that, despite sterility, genotypes could be deduced for as many wild type males as were present in the randomly chosen 100.

Genotypes corresponding to two of the four phenotypic classes could be determined without further testing. Flies which exhibited vein interruptions and normal eyes were $+^3/ci\ ey^R$. Those showing both mutant characteristics were $ci\ ey^R/ci\ ey^R$. The few flies which were eyeless only were progeny-tested and proved to be $ci\ ey^R/ci\ ey^R$ as expected. It should be noted that *ci* homozygotes do not demonstrate 100 percent penetrance, while ey^R/ey^R are always eyeless.

Flies which were wild type in appearance could have been of various genetic constitutions. Genotypes were determined by mating these normal males singly to tester $ci\ ey^R/ci\ ey^R$ females. This process was simple as far as cages 7 and 8 were concerned. In cage 9, however, due to the presence of three alleles, five different genotypes could contribute to the class of normal flies. Following the usual progeny testing, genotypes of male parents were assigned on the following basis. If all sons were wild type, the tested male was $+^4/+^4$. When wild type and *ci*, eyeless sons appeared, the male parent was $+^4/ci\ ey^R$. If three classes of male progeny were found, namely normal, *ci* eyeless, and also *ci* alone, the father was listed as $+^3/ci\ ey^R$. Two kinds of males produced normal and *ci*, noneyeless sons. These were the $+^4/+^3$ and $+^3/+^3$ males. However, the expected frequencies of the two types of male progeny were quite different for each of the males. A $+^3/+^3$ male produced sons, 77 to 100 percent of which were *ci* in appearance, while a maximum of 57 percent of the sons of a $+^4/+^3$ male would be so marked.

RESULTS

Cage experiments involving $+^4$ and $+^3$

The results obtained in all three experimental populations in which $+^4$ and $+^3$ were the only genes present at the *ci* locus indicate a marked superiority for the $+^4$-carrying chromosome. Regardless of the frequencies of the two types at the outset of the experiment, $+^4$ increased with a concomitant decrease of $+^3$ (see Tables 1, 2, and 3). In cage 1, where the initial frequencies were equal, no striking changes were observed in the samples of the first three months. The allele $+^4$ increased to 0.7 by the fifth month and thereafter rose substantially to 0.915 at the termination of the population at fifteen months. It is obvious that the frequencies of the three genotypes in the last six samples differed significantly from the values of 0.25 $+^4/+^4$, 0.50 $+^4/+^3$, and 0.25 $+^3/+^3$ expected if no selective differences existed between $+^4$ and $+^3$. From the gametic frequencies of each sample were calculated the proportions of the three genotypes expected on the basis of the Hardy-Weinberg formulation. Deviations between observed and expected were analyzed by the chi-square method with one degree of freedom for each test. An excess of heterozygotes was observed in eight of the nine samples, but none of the chi-squares was significant at the five percent level.

In cage 3, $+^4$ increased from the initial value of 0.3 to 0.403 in the first month's sample. Proportions of the three genotypes differed significantly ($\chi^2 = 10.45$, P

TABLE 1

Zygotic and gametic frequencies of $+^4$ and $+^3$ chromosomes in samples of population cage 1 started December 22, 1954 with 200 $+^4$ and 200 $+^3$ unmated flies; $p(+^4) = 0.5$, $q(+^3) = 0.5$

Date of sample		Zygotic frequencies				Gametic frequencies in percent	
		$+^4/+^4$	$+^4/+^3$	$+^3/+^3$	χ^2	$+^4$	$+^3$
January	Obs.	22	59	19	...	51.5	48.5
21	Exp.	26.5	50	23.5	3.25	...	...
1955	Diff.	–4.5	9	–4.5	...	...	...
February	Obs.	25	54	16	...	54.7	45.3
21	Exp.	28.4	47.1	19.5	2.06	...	...
	Diff.	–3.4	6.9	–3.5	...	...	...
March	Obs.	29	49	22	...	53.5	46.5
21	Exp.	28.6	49.8	21.6	0.02	...	...
	Diff.	0.4	–0.8	0.4	...	...	...
May	Obs.	47	46	7	...	70.0	30.0
17	Exp.	49	42	9	0.91	...	...
	Diff.	–2	4	–2	...	...	...
June	Obs.	49	45	6	...	71.5	28.5
21	Exp.	51.1	40.8	8.1	0.97	...	...
	Diff.	–2.1	4.2	–2.1	...	...	...
August	Obs.	58	37	5	...	76.5	23.5
20	Exp.	58.5	36	5.5	0.08	...	...
	Diff.	–0.5	1	–0.5	...	...	...
October	Obs.	64	33	3	...	80.5	19.5
25	Exp.	64.8	31.4	3.8	0.26	...	...
	Diff.	–0.8	1.6	–0.8	...	...	...
December	Obs.	79	20	1	...	89.0	11.0
13	Exp.	79.2	19.6	1.2	0.05	...	...
	Diff.	–0.2	0.4	–0.2	...	...	...
March	Obs.	83	17	0	...	91.5	8.5
31	Exp.	83.7	15.6	0.7	0.90	...	...
1956	Diff.	–0.7	1.4	–0.7	...	...	...

< 0.01) from the values of 0.49 $+^3/+^3$, 0.42 $+^3/+^4$, and 0.09 $+^4/+^4$ that would have been expected if no change in gene frequency had occurred and Hardy-Weinberg equilibrium were present. Further increase of $+^4$ proceeded slowly until the fifth month, at which time the sample estimated $+^4$ at 58.0 percent. The next major change occurred between the eighth and tenth months, $+^4$ growing from 64.3 to 76.0 percent. A final assay of the population at the end of fifteen months placed $+^4$ at 83.0 percent. Eight of the eleven samples contained more heterozygotes than expected on Hardy-Weinberg grounds. Four of these eight had chi-square values with probabilities between 0.01 and 0.05.

No prominent changes were observed in the first two samples of cage 5. The frequency of $+^4$ rose from an initial 70 percent to 78.5 percent in the sample taken in the fourth month. Frequencies of the three genotypes differed significantly at the 0.01 level from the values expected if there had been no change in p

and if the Hardy-Weinberg law applied. Flies homozygous for $+^3$ were infrequent in all of the samples and were absent from the final samples taken at the eleventh and fifteenth months. The last estimate of the frequency of $+^4$ in the population was 94.5 percent. None of the samples of cage 5 showed significant deviations from Hardy-Weinberg equilibrium. Five of them contained an excess, and three a deficiency of heterozygotes.

TABLE 2

Zygotic and gametic frequencies of $+^4$ and $+^3$ chromosomes in samples of population cage 3 started December 30, 1954 with 60 $+^4$ and 140 $+^3$ unmated flies;
$p(+^4) = 0.3$, $q(+^3) = 0.7$

Date of sample		Zygotic frequencies				Gametic frequencies in percent	
		$+^4/+^4$	$+^4/+^3$	$+^3/+^3$	χ^2	$+^4$	$+^3$
January	Obs.	16	51	36	...	40.3	59.7
31	Exp.	16.7	49.6	36.7	0.06	...	...
1955	Diff.	–0.7	1.4	–0.7	...	...	...
February	Obs.	12	59	29	...	41.5	58.5
28	Exp.	17.2	48.6	34.2	4.59*	...	...
	Diff.	–5.2	10.4	–5.2	...	...	...
March	Obs.	18	52	30	...	44.0	56.0
30	Exp.	19.4	49.2	31.4	0.30	...	...
	Diff.	–1.4	2.8	–1.4	...	...	...
May	Obs.	33	50	17	...	58.0	42.0
21	Exp.	33.6	48.8	17.6	0.07	...	...
	Diff.	–0.6	1.2	–0.6	...	...	...
June	Obs.	27	61	12	...	57.5	42.5
22	Exp.	33	49	18	6.04*	...	...
	Diff.	–6	12	–6	...	...	...
July	Obs.	36	56	8	...	64.0	36.0
23	Exp.	41	46	13	4.70*	...	...
	Diff.	–5	10	–5	...	...	...
August	Obs.	36	57	7	...	64.5	35.5
24	Exp.	41.6	45.8	12.6	6.05*	...	...
	Diff.	–5.6	11.2	–5.6	...	...	...
October	Obs.	58	36	6	...	76.0	24.0
26	Exp.	57.7	36.6	5.7	0.03	...	...
	Diff.	0.3	–0.6	0.3	...	...	...
December	Obs.	65	30	7	...	78.0	22.0
14	Exp.	60.8	34.4	4.8	1.64	...	...
	Diff.	2.2	–4.4	2.2	...	...	...
January	Obs.	69	29	2	...	83.5	16.5
16	Exp.	69.7	27.6	2.7	0.26	...	...
1956	Diff.	–0.7	1.4	–0.7	...	...	...
March	Obs.	70	26	4	...	83.0	17.0
30	Exp.	68.9	28.2	2.9	0.62	...	...
	Diff.	1.1	–2.2	1.1	...	...	...

* Significant at 0.05 level.

TABLE 3

Zygotic and gametic frequencies of $+^4$ and $+^3$ chromosomes in samples of population cage 5 started January 12, 1955 with 140 $+^4$ and 60 $+^3$ unmated flies;
$p(+^4) = 0.7$, $q(+^3) = 0.3$

Date of sample		Zygotic frequencies				Gametic frequencies in percent	
		$+^4/+^4$	$+^4/+^3$	$+^3/+^3$	χ^2	$+^4$	$+^3$
February	Obs.	55	38	7	...	74.0	26.0
15	Exp.	54.8	38.4	6.8	0.02	...	...
1955	Diff.	0.2	–0.4	0.2	...	...	...
March	Obs.	54	41	5	...	74.5	25.5
13	Exp.	55.5	38.1	6.5	0.62	...	...
	Diff.	–1.5	2.9	–1.5	...	...	...
May	Obs.	60	37	3	...	78.5	21.5
26	Exp.	61.6	33.8	4.6	0.86	...	...
	Diff.	–1.6	3.2	–1.6	...	...	...
June	Obs.	61	35	4	...	78.5	21.5
28	Exp.	61.6	33.8	4.6	0.15	...	...
	Diff.	–0.6	1.2	–0.6	...	...	...
August	Obs.	69	28	3	...	83.0	17.0
31	Exp.	69	28.1	2.9	0.00	...	...
	Diff.	0	–0.1	0.1	...	...	...
October	Obs.	74	22	4	...	85.0	15.0
27	Exp.	72.3	25.5	2.3	1.97	...	...
	Diff.	1.7	–3.5	1.7	...	...	...
December	Obs.	82	18	0	...	91.0	9.0
15	Exp.	82.8	16.4	0.8	0.98	...	...
	Diff.	–0.8	1.6	–0.8	...	...	...
April	Obs.	89	11	0	...	94.5	5.5
24	Exp.	89.3	10.4	0.3	0.34	...	...
1956	Diff.	–0.3	0.6	–0.3	...	...	...

Cage experiments involving $+^4$, $+^3$ and ci ey^R

Flies homozygous for the fourth chromosome recessives, *ci* and *ey^R* were the most frequent types present initially in three experimental populations raised at 25°C. In cage 7 the original frequency was 0.3 for $+^3$ and 0.7 for *ci ey^R*. Nevertheless, in the first sample only a single *ci ey^R/ci ey^R* male was present and the frequency of $+^3$ had risen to 0.78! (See Table 4). The proportions of the three genotypes differed greatly ($\chi^2 > 300$) from the numbers expected from the introduced chromosomal frequencies. The four subsequent samples deviated to an even greater extent. (χ^2 ranged from > 450 to > 750.) Estimates of the occurrence of $+^3$ varied from 95.0 percent to 91.5 percent in the last three samples, and *ci ey^R* homozygotes were absent. Hardy-Weinberg quilibrium for each of the samples was tested by the chi-square method. Probability values ranged from between 0.05 and 0.02 for sample one, to larger than 0.05 for the remaining samples. Heterozygotes were more numerous than expected in each sample.

A similar rapid decrease of the *ci ey*^{R} chromosome occurred in cage 8. Here it was placed in competition with the $+^4$ type, the latter comprising 30 percent of the starting population. As recorded in Table 5, in one month $+^4$ rose to 58.5 percent. The next sample estimated $+^4$ at 80.5 percent and continued gains were observed until a high point of 95.5 percent was attained in the ninth month. Genotypic frequencies of all six samples differed significantly from the proportions calculated from the original gametic values. Chi-square tests for Hardy-Weinberg equilibrium disclosed that the initial sample deviated significantly at the one percent level; the second sample had a probability between 0.02 and 0.05; and the last four samples had P values above 0.05. No consistency among the samples in the ratio of observed and expected heterozygotes was noted.

The population with the most complex initial genetic composition occupied cage 9. All three chromosomal types were introduced in the proportions of 0.6 *ci ey*^{R}, 0.2 $+^3$, and 0.2 $+^4$. Table 6 presents the data of seven samples of this population covering a period of ten months. The following trends are manifested in the samples:

(1) There was an immediate and sustained decrease in the frequency of the *ci ey*^{R} chromosome culminating in its absence in the final sample. Mainly responsible for this decline was the virtual absence, from the beginning, of homozygous *ci ey*^{R} flies. In addition, after registering very highly in the first sample, $+^3$/*ci ey*^{R} heterozygotes diminished quickly to two percent or less in

TABLE 4

Zygotic and gametic frequencies of $+^3$ *and* ci ey^R *chromosomes in samples of population cage 7 started July 13, 1955 with 60* $+^3$ *and 140* ci ey^R *unmated flies;* $p(+^3) = 0.3$, $q(\text{ci ey}^R) = 0.7$

Date of sample		Zygotic frequencies				Gametic frequencies in percent	
		$+^3/+^3$	$+^3/ci\ ey^R$	$ci\ ey^R/ci\ ey^R$	χ^2	$+^3$	*ci ey*^{R}
August	Obs.	57	42	1	...	78.0	22.0
12	Exp.	60.8	34.4	4.8	4.95*	...	...
1955	Diff.	–3.8	7.6	–3.8	...	...	...
September	Obs.	70	29	1	...	84.5	15.5
14	Exp.	71.4	26.2	2.4	1.12	...	...
	Diff.	–1.4	2.8	–1.4	...	...	...
October	Obs.	90	10	0	...	95.0	5.0
18	Exp.	90.3	9.4	0.3	0.28	...	...
	Diff.	–0.3	0.6	–0.3	...	...	...
November	Obs.	85	15	0	...	92.5	7.5
17	Exp.	85.6	13.8	0.6	0.69	...	...
	Diff.	–0.6	1.2	–0.6	...	...	...
December	Obs.	83	17	0	...	91.5	8.5
8	Exp.	83.7	15.6	0.7	0.80	...	...
	Diff.	–0.7	1.4	–0.7	...	...	...

* Significant at 0.05 level.

TABLE 5

Zygotic and gametic frequencies of $+^4$ and ci ey^R *chromosomes in samples of population cage 8 started July 13, 1955 with 60 $+^4$ and 140* ci ey^R *unmated flies;* $p(+^4) = 0.3$, $q(ci\ ey^R) = 0.7$

Date of sample		Zygotic frequencies				Gametic frequencies in percent	
		$+^4/+^4$	$+^4/ci\ ey^R$	$ci\ ey^R/ci\ ey^R$	χ^2	$+^4$	$ci\ ey^R$
August	Obs.	24	69	7	...	58.5	41.5
19	Exp.	34.2	48.6	17.2	17.61†	...	...
1955	Diff.	−10.2	20.4	−10.2	...	...	...
September	Obs.	68	25	7	...	80.5	19.5
16	Exp.	64.8	31.4	3.8	4.16*	...	...
	Diff.	3.2	−6.4	3.2	...	...	...
October	Obs.	77	19	4	...	86.5	13.5
19	Exp.	74.8	23.4	1.8	3.58	...	...
	Diff.	2.2	−4.4	2.2	...	...	...
November	Obs.	87	11	2	...	92.5	7.5
18	Exp.	85.6	13.8	0.6	3.85	...	...
	Diff.	1.4	−2.8	1.4	...	...	...
December	Obs.	86	14	0	...	93.0	7.0
9	Exp.	86.5	13.0	0.5	0.58	...	...
	Diff.	−0.5	1.0	−0.5	...	...	...
April	Obs.	91	9	0	...	95.5	4.5
26	Exp.	91.2	8.6	0.2	0.22	...	...
1956	Diff.	−0.2	0.4	−0.2	...	...	...

* Significant at 0.05 level.
† Significant at 0.01 level.

samples 4, 5 and 6. The $+^4/ci\ ey^R$ flies approximated ten percent in the first five samples, but decreased to one percent in the sixth sample.

(2) In contrast to the steady decline of the *ci* ey^R chromosome, $+^4$, which measured 23.5 percent in sample one, climbed consistently until it reached 83.5 percent in the seventh sample. A single genotype, $+^4/+^4$, gained regularly in each sample following its low point in the initial one. The heterozygote, $+^4/+^3$, increased to about 40 percent in the third sample, and maintained this frequency until sample 7, when it registered at 25 percent. As mentioned above, $+^4/ci\ ey^R$ remained fairly constant until its drop in the final samples.

(3) The $+^3$ chromosome, which varied between 40.0 and 47.0 percent, was the most frequent type for the first three samples. It was overtaken in the fourth month and thereafter decreased to a low of 16.5 percent by the seventh sample. Most of the remaining $+^3$ chromosomes were found in the $+^4/+^3$, since the $+^3/ci\ ey^R$ heterozygotes declined drastically subsequent to their high marks in early samples, and the $+^3/+^3$ fluctuated from a low of three percent to a high of about 20 percent and finally four percent in the final sample.

Inspection of the data reveals that none of the samples approached the proportions of the six genotypes expected on the basis of the introduced frequencies

TABLE 6

Zygotic and gametic frequencies of $+^4$, $+^3$, *and* ci ey^R *chromosomes in samples of population cage 9 started July 15, 1955 with 40* $+^4$, *40* $+^3$, *and 120* ci ey^R *unmated flies;* $p(+^4)=0.2$, $q(+^3)=0.2$, $r(ci\ ey^R)=0.6$

Date of sample		Zygotic frequencies							Gametic frequencies in percent		
		$+^4/+^4$	$+^4/+^3$	$+^4/ci\ ey^R$	$+^3/+^3$	$+^3/ci\ ey^R$	$ci\ ey^R/ci\ ey^R$	χ^{2*}	$+^4$	$+^3$	$ci\ ey^R$
August	Obs.	6	21	14	3	53	3	...	23.5	40.0	36.5
23	Exp.	5.5	18.8	17.2	16	29.2	13.3	38.90†	...	...	...
1955	Diff.	0.5	2.2	–3.2	–13	23.8	–10.3	...	...	...	...
September	Obs.	15	29	8	17	31	0	...	33.5	47.0	19.5
27	Exp.	11.2	31.5	13.1	22.1	18.3	3.8	17.27†	...	...	...
	Diff.	3.8	–2.5	–5.1	–5.1	12.7	–3.8	...	...	...	...
October	Obs.	18	36	7	20	14	5	...	39.5	45.0	15.5
20	Exp.	15.6	35.5	12.3	20.3	13.9	2.4	5.50	...	...	...
	Diff.	2.4	0.5	–5.3	–0.3	0.1	2.6	...	...	...	...
November	Obs.	22	44	10	22	2	0	...	49.0	45.0	6.0
19	Exp.	24	44	5.8	20.4	5.4	0.4	5.74	...	...	...
	Diff.	–2	0	4.2	1.6	–3.4	–0.4	...	...	...	...
December	Obs.	33	42	11	11	2	1	...	59.5	33.0	7.5
10	Exp.	35.5	39.2	8.9	10.9	4.9	0.6	2.94	...	...	...
	Diff.	–2.5	2.8	2.1	0.1	–2.9	0.4	...	...	...	...
January	Obs.	54	37	1	7	1	0	...	73.0	26.0	1.0
15	Exp.	53.3	38	1.5	6.7	0.5	0	0.72	...	...	...
1956	Diff.	0.7	–1	–0.5	0.3	0.5	0	...	...	...	...
April	Obs.	71	25	0	4	0	0	...	83.5	16.5	0.0
25	Exp.	69.7	27.6	0	2.7	0	0	1.29	...	...	...
	Diff.	1.3	–2.6	0	1.3	0	0	...	...	...	...

* Three degrees of freedom for each test of Hardy-Weinberg equilibrium except for final sample.
† Significant at 0.01 level.

of the three types of chromosomes. While the first two samples were not in Hardy-Weinberg equilibrium, the remaining ones did not deviate from such equilibrium by more than would be expected to occur by chance ($P > 0.10$).

At the time the final samples were taken, census counts were attempted of the populations inhabiting cages 1 and 9. In neither case could all the flies be captured, since the aspiration method became less efficient as the number of flies remaining in the cage was reduced. The flies captured in cage 1 numbered 3,784, while 3,854 were counted from cage 9. No deviation from a 1:1 sex ratio was observed in random samples of each final count classified according to sex. For cage 1 there were 802 females and 791 males; cage 9, 498 females and 484 males.

DISCUSSION

A clarification of the use of the terms isoallele and chromosome appears advisable at this time prior to the discussion of the cage experiments. Granting that $+^4$ and $+^3$ are allelic, what is the relationship between their difference in the degree of dominance over the *ci* allele and the results of competition between chromosomes carrying them? Knowledge of this relationship is important because crossing over occurs extremely infrequently, if at all, between the fourth chromosomes under the cytologically normal conditions which obtained in the experimental populations. We have then, in all likelihood, two chromosomes retaining their individuality throughout the experiment. A claim that varying alleles at the *ci* locus comprise the sole genetic difference between these two wild chromosomes would go beyond the facts. Perhaps other genic variations along the lengths of the two chromosomes are responsible for the inequalities in adaptive value found. Nevertheless, barring the possibility of close-linked dominant modifiers, the chromosomes have been shown, by making them heterozygous with the *ci ey*^{R} chromosome, to be different at the locus of cubitus interruptus while genetic dissimilarities at any other loci are as yet undetected.

For purposes of conciseness the terms $+^4$ chromosome and $+^3$ chromosome, or even simply $+^4$ and $+^3$ are used in connection with the cage experiments. This is not meant to imply that selective differences between the chromosomes are due to the isoalleles alone. The isoalleles may rank in importance anywhere from nonadaptive but experimentally useful "tags" to comprising the causative agent of any fitness differential. That this latter view is not unreasonable is indicated by three considerations. First it is to be recalled that all flies were presumably isogenic for chromosomes I, II and III. Therefore, the basis for any observed selective advantages must be sought in the fourth chromosome. Secondly, the fourth chromosome is very small in relation to the rest of the genome. Since it contains presumably few genes, any known difference, in this case at the *ci* locus, gains increased significance. Thirdly, the order of fitness parallels the degree of dominance and hence the order of morphological effects, the isoallele with weaker dominance being the less fit.

Two fourth chromosomes, one carrying the $+^3$ allele, the other $+^4$, were in competition in four cages. Final samples of all the experimental populations

revealed that, without exception, $+^4$ had increased significantly over its original frequency. There can be little doubt that selection has been the force responsible for these changes in population structure. The other known agencies which may alter gene frequencies can be dismissed from our considerations. An inordinately high mutation rate of $+^3 \longrightarrow +^4$ would be necessary to account for the changes noted. Migration obviously was not involved and due to the large size of the population, the effect of genetic drift is negligible. CROW and MORTON (1955) estimate that the effective population number (the progenitors of the next generation) lies between 0.7 and 0.95 of the actual number in laboratory populations of Drosophila unless conditions are unusual. Even granting that the actual number of adults is as low as 3,000, this would place the effective number at over 2,000, a figure large enough to allow us to ignore the influence of drift with a minimum of hesitation. Chance may have operated significantly in the early periods of the experiments before the populations increased to their large and fairly constant sizes, but drift was inconsequential as far as end results were concerned.

The parallelism between cages and the magnitude of the changes argue for natural selection as the agent responsible for the increase of $+^4$ at the expense of $+^3$. In addition, mathematical analyses of three of the selection experiments (cages 1, 3 and 5) were undertaken to obtain quantitative estimates of the adaptive values of the three pertinent genotypes, $+^4/+^4$, $+^4/+^3$ and $+^3/+^3$. The method employed is that of DOBZHANSKY and LEVENE (1951).

Since this method is clearly presented in the paper cited, there will be no attempt to repeat the details. However, the formulae used in obtaining the figures in Table 7 and in plotting the graphs in Figures 2, 3 and 4 are as follows:

The adaptive value of the heterozygote, $+^4/+^3$, is taken at 1. The adaptive values of the two homozygotes, $+^3/+^3$ and $+^4/+^4$, are expressed as W_1 and W_2, respectively.

$$W_2 = a + b\,W_1 \qquad \text{where}$$

$$a = \left(\frac{X_0}{X_1}\right)^{1/t} - X_0 \qquad \text{and}$$

$$b = X_0\left(\frac{X_0}{X_1}\right)^{1/t} \qquad \text{where } X_0 \text{ and } X_1 \text{ are ratios}$$

of the frequencies of $+^3$ to $+^4$ estimated for any two successive samples and t is the time in generations with the length of a generation in a cage at 25°C taken as 15 days.

The various X's each have a variance which is calculated by the formula:

$$X^2 = \frac{X(1+X)^2}{2n}$$

where n equals the number of flies tested in each sample. From this variance the standard error, σ_X, of each X is determined, and it is assumed that the true value of the ratio of $+^3$ to $+^4$ in any sample ($\overline{X}$) should lie within the range $X \pm 1.96\,\sigma_X$ 95 percent of the time.

TABLE 7

Results of cages 1, 3 and 5 used in calculations of adaptive values. See text for details

Period number	Time elapsed in days	Generation time t	Flies in sample n	Ratio $\frac{+^3}{X} : +^4$	Standard error σX	Coefficients for finding adaptive values	
						a	b
			Cage 1				
0	0	0	...	1.00	0	...	...
1	30	2.0	100	.94	.133	.03	1.03
2	32	2.1	95	.83	.118	.12	1.00
3	28	1.9	100	.87	.123	.15	.81
4	54	3.6	100	.43	.066	1.03	1.65
5	35	2.3	100	.40	.063	.60	.44
6	60	4.0	100	.31	.052	.66	.42
7	66	4.4	100	.24	.043	.75	.33
8	49	3.2	100	.12	.027	1.00	.30
9	108	7.2	100	.09	.024	.92	.12
			Cage 3				
0	0	0	...	2.33	0	...	...
1	31	2.1	103	1.48	.211	–1.08	2.91
2	28	1.9	100	1.41	.202	–0.46	1.51
3	31	2.1	100	1.27	.180	–0.36	1.58
4	51	3.4	100	.72	.103	–0.09	1.50
5	32	2.1	100	.74	.106	.27	0.71
6	31	2.1	100	.56	.082	.41	.85
7	32	2.1	100	.55	.081	.45	.57
8	63	4.2	100	.32	.053	.60	.63
9	49	3.2	100	.28	.048	.72	.33
10	33	2.2	100	.20 (.198)	.038	.91	.33
11	73	4.9	100	.20 (.205)	.038	.80	.20
			Cage 5				
0	0	0	...	0.43	0	...	...
1	34	2.2	100	.35	.057	.66	.47
2	26	1.8	100	.34	.055	.67	.36
3	74	5.0	100	.27	.047	.71	.36
4	33	2.2	100	.27	.047	.73	.27
5	64	4.2	100	.20	.038	.80	.29
6	57	3.8	100	.18	.035	.83	.21
7	49	3.2	100	.10	.025	1.02	.22
8	130	8.6	100	.06	.018	.96	.11

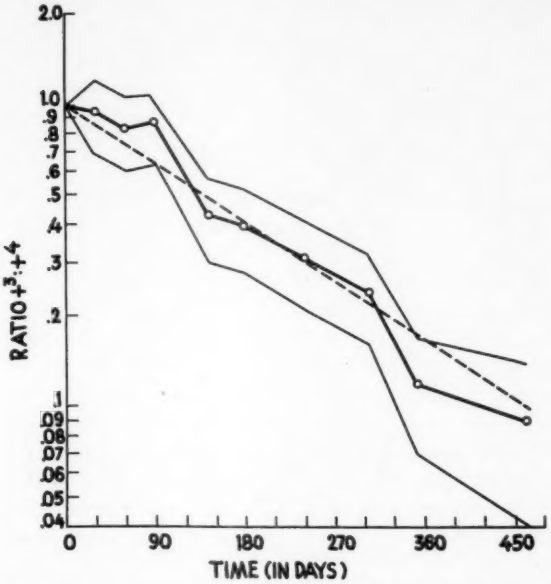

FIGURE 2.—Results of cage 1. Vertical axis gives ratio of $+^3$ to $+^4$. Horizontal axis gives time in days. Circles denote sample values, upper and lower solid lines are 95 percent confidence limits for true values. Dotted line gives theoretical changes if the following adaptive values are true: W_1 $(+^3/+^3) = 0.95$ and W_2 $(+^4/+^4) = 1.1$.

Figure 2 presents a semilogarithmic plot of X against time for cage 1. The middle solid line connects the values of X (circled points) and the two outer solid lines connect values of $X \pm 1.96\ \sigma_X$. The dotted line gives the theoretical changes in X expected if adaptive values are $W_1(+^3/+^3) = 0.95$; $W_2(+^4/+^4) = 1.1$. With the exception of a small segment, the entire theoretical curve falls within the 95 percent confidence limits indicating that the W's are good approximations. There is no need to postulate any change in adaptive values with time since constant W's fit well throughout the experiment. A trial and error method demonstrated that curves based on other values of W_1 and W_2 did not fit as well as those used in Figure 3. Analyses of the results of cages 3 and 5 gave the following best estimates of adaptive values:

Cage 3 $W_1 = 0.90$; $W_2 = 1.1$
Cage 5 $W_1 = 0.95$; $W_2 = 1.05$

While considerably less than five percent of the theoretical curve plotted for cage 3 (Figure 3) lies outside of the 95 percent confidence limits, it should be noted that this portion is at the end of the curve. Based on the adaptive values of 0.90 $(+^3/+^3)$ and 1.1 $(+^4/+^4)$ one would expect the ratio of $+^3 : +^4$ to be closer

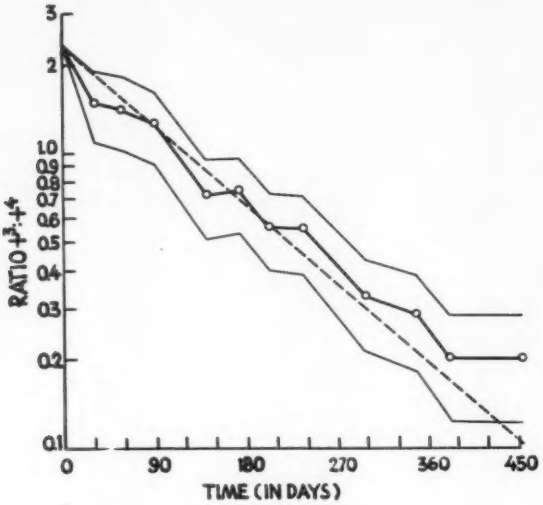

FIGURE 3.—Results of cage 3. Legend as in Figure 2 with this exception, $W_1 = 0.9$ and $W_2 = 1.1$.

to 0.1 than the value of 0.2 calculated from the last sample. Nevertheless, the estimated adaptive values provide a curve of good fit and they can be considered the best W's available. The fact that $+^3$ scored higher than expected in the final sample may be due to sampling errors.

The whole theoretical curve for cage 5 (Figure 4) falls inside the 95 percent confidence area. The difference between the adaptive values of the two homozygous types is least in cage 5, (1.05 to 0.95). This is a result of the fact that the $+^4$ chromosome made the smallest gains in cage 5, (0.7 to 0.945), in the fifteen month period that all three cages were followed. The greatest gain, (0.3 to 0.83), and largest difference in adaptive values, (1.1 to 0.9), were observed in cage 3, with cage 1 falling in the middle (0.5 to 0.915) with W's of 1.1 to 0.95.

Three things are apparent from the analyses of these three cage experiments.

(1) The adaptive value of the $+^4/+^4$ homozygote is consistently greater than that of the heterozygote $+^4/+^3$ which in turn surpasses that of the $+^3/+^3$ genotype.

(2) There are considerable differences between the adaptive values of the three genotypes within each population. The discrepancy between 1.05 and 0.95 may not seem very large at first glance. However, when one considers that this means that in every generation the $+^4/+^4$ genotype reproduces 105 offspring for each 95 the other homozygote leaves, the evolutionary significance of the difference in adaptive values is appreciated.

(3) It appears likely that the adaptive values have remained unchanged throughout the course of the experiments.

It can be concluded from a study of successive samples as well as from the mathematical analyses of the selection experiments that no equilibrium between $+^4$ and $+^3$ has been reached in any of the populations. Instead of becoming stabilized at some frequency the $+^4$ chromosome is apparently continuing to increase in each cage at the termination of the experiments. Possibly a balanced state would have been attained at some future time when the frequencies of the two chromosomes were even more disproportionate than the values of 0.945 and 0.055 estimated in cage 5. It seems more probable, however, that $+^3$ is destined to become increasingly rare and perhaps eventually lost. The lack of heterotic heterozygotes and the decided inferiority of the $+^3/+^3$ homozygote are responsible for the decline of $+^3$. (The concept of heterosis used here refers only to the adaptive superiority of heterozygotes over homozygotes. The author is aware that the term heterosis may signify different things to different geneticists. Adaptive heterosis or euheterosis (DOBZHANSKY 1950) exists when an organism, heterozygous for a single gene or a gene complex, enjoys a selective advantage over either homozygote and thereby maintains a condition of balanced polymorphism in the population.) There are slight indications that the $+^4/+^3$ genotype may be superior in viability to either homozygote. Of the 28 samples of cages 1,

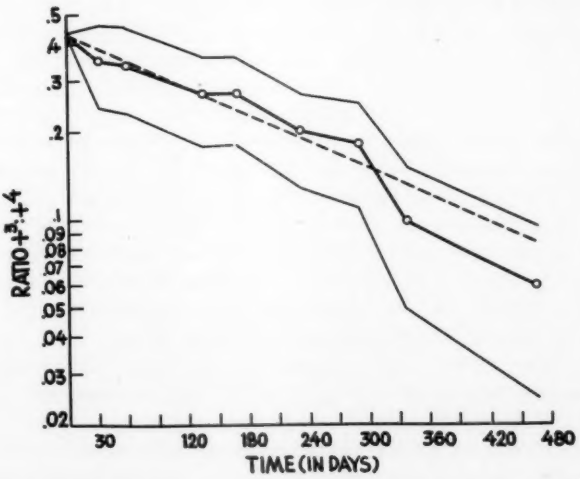

FIGURE 4.—Results of cage 5. Legend as in Figure 2 with this exception, $W_1 = 0.95$ and $W_2 = 1.05$.

3 and 5, 19 contained heterozygotes in excess of the frequency expected on the assumption that each sample was in Hardy-Weinberg equilibrium. However, increased viability is not synonymous with adaptive superiority. The mathematical analysis of the selection experiments reveals that the $+^4/+^4$ homozygote has a higher adaptive value than the heterozygote.

The observed absence of heterosis is open to more than one interpretation. Since the two chromosomes in question have perhaps never coexisted in a natural population, there has been no opportunity for coadapted gene complexes to arise. DOBZHANSKY (1950) has reported that inversion heterozygotes in *D. pseudoobscura* which contain two chromosomes of different geographical origin usually show no heterosis. On the other hand, DOBZHANSKY and LEVENE (1951) postulate that heterosis may arise through interaction between the gene complexes preserved in the inversion heterozygote and genes present on other chromosomes. In the *pseudoobscura* experiments, flies with many different genetic backgrounds are used so that presumably a rich supply of genic variability is available in the population. Out of the numerous possible combinations perhaps certain ones, in conjunction with the inversion heterozygote, confer adaptive superiority. The isogeneity of chromosomes I, II and III which characterized the populations in the present study made for a minimum of genetic variability. Apparently no genotypic combinations, involving these three chromosomes, when taken jointly with the $+^3/+^4$ heterozygote were favored by selection. (In the event that chromosomes I, II and III were homozygous at all loci, there would of course have been but a single combination. The plural is used on the assumption that certain loci may be heterozygous.)

The presence of an additional fourth chromosome type (*ci ey*^{R}) in the experimental population raised in cage 9 seemingly had little major effect upon the relationship of $+^4$ and $+^3$. Initially the two wild chromosomes were present in equal numbers. At the conclusion of the experiment in the tenth month $+^4$ had increased to 83.5 percent and $+^3$ had declined to 16.5 percent. The differential is not unlike that measured in cage 1 ten months after the population was started with $+^4$ and $+^3$ in equal frequencies. In cage 9 the three genotypes, $+^4/+^4$, $+^4/+^3$ and $+^3/+^3$ comprised 100 percent of the final sample. The *ci ey*^{R} chromosome is, as might be expected, of very poor viability and, for the most part, assumes a minor role in the genetic composition of the population. An exception in early samples is found in the $+^4/$*ci ey*^{R} heterozygote which temporarily stabilized at approximately ten percent prior to the last two samples. It is interesting to compare the fate of this genotype within cage 9 and cage 8. In cage 8 $+^4$ and *ci ey*^{R} alone were in competition and at the end of ten months the *ci ey*^{R} frequency was reduced to 0.045. All of the *ci ey*^{R} chromosomes were present in heterozygous $+^4/$*ci ey*^{R} flies, the homozygotes being absent in the last samples. Whether this chromosome would have been eventually supplanted entirely or would have remained in the population due to a heterotic heterozygote cannot be determined. On the basis of the comparison of cages 8 and 9, it is tentatively

concluded that the $+^4/ci\ ey^R$ karyotype is more likely to remain in a population in which $+^3$ is absent.

A possible example of an adaptively superior heterozygote is the $+^3/ci\ ey^R$ genotype found in cage 7. The $+^3$ chromosome rose rapidly from an initial value of 30 percent to 95 percent in the third monthly sample. Instead of continuing to decline, $ci\ ey^R$ increased from the 5 percent observed in the third sample to 8.5 percent in sample 5. This increase, however, may be due to errors of sampling instead of reflecting an approach to equilibrium. No $ci\ ey^R/ci\ ey^R$ flies were seen in the last three samples, the $ci\ ey^R$ chromosome being maintained therefore only in the heterozygous condition. Under the different genetic conditions of cage 9, complicated by the presence of a competing $+^4$ chromosome, the $+^3/ci\ ey^R$ genotype followed a vastly different pattern of evolution. Present at a very high frequency in the first sample, it declined quickly. The relationship of $+^3$ and $ci\ ey^R$ was therefore affected to a large degree by the $+^4$ chromosome.

The results of experiments involving competition between ci^+ isoalleles and the *ci* allele are not different from those which have been usually observed in studies of wild genes versus mutant alleles (see for example, MERRELL and UNDERHILL 1956). Unfortunately, the presence of the ey^R gene (for technical reasons) weakens the case against *ci*, since ey^R is doubtless inferior to the ey^+ gene present in both the $+^4$ and $+^3$ chromosomes. The chief contribution of the $ci\ ey^R$ chromosome to this study is found in the fact that its presence in cage 9 created a genetic population structure different from that in the three other cages in which $+^4$ and $+^3$ competed. Perhaps new levels upon which selection could operate were introduced in this manner. Nevertheless, the ultimate fates of $+^4$ and $+^3$ were not altered.

Under the genetical and environmental conditions which were established in the experimental populations studied, the difference in wild type alleles at the *ci* locus served a function more important than one of merely increasing heterozygosity within the population. An evolutionary change of gradual substitution and possible fixation of the more favorable allele was noted. Although it is probable that the difference, in terms of genic structure and physiology, between two wild type isoalleles, is less than that between a normal gene and its mutant allele, the results of this investigation demonstrate that the selective difference between two isoalleles can be striking.

Further experimentation is required before one can conclude that $+^3$ and similar ci^+ alleles are adaptively inferior under other laboratory conditions or in nature. Supporting evidence, however, has come from a large scale survey of wild populations, the details of which will be published elsewhere. The author has tested phenotypically normal flies, from both laboratory strains and the wild, for isoalleles at the *ci* locus. Only a single isoallele of the $+^3$ type was uncovered, although over 30 wild alleles were examined as heterozygotes with the mutant allele *ci*. If selection pressure against $+^3$ type isoalleles is similar, in nature and

in laboratory stocks, to that found in the cage populations studied, one would expect such alleles to be rare.

Several lines of investigation to be pursued include placing $+^4$ and $+^3$ in cage competition again with a more heterogeneous genetic milieu replacing the high degree of isogeneity employed previously. In addition, a newly discovered isoallele which is equivalent to $+^3$, as measured by its dominance over *ci*, will be subjected to selection experiments to determine whether or not its adaptive value resembles that of $+^3$.

SUMMARY

1. Laboratory studies involving two wild type isoalleles at the fourth chromosome locus of cubitus interruptus in *Drosophila melanogaster* were carried out. The two alleles can be distinguished by their different degrees of dominance with respect to the mutant allele *ci*. One wild type allele, $+^4$, is completely dominant over *ci* while the other allele, $+^3$, allows a majority of flies with the genotype $+^3/ci$ to express the phenotype of interrupted wing veins which characterizes the *ci*/*ci* genetic constitution.

2. The population cage technique was utilized to determine the relative selective advantage of a chromosome carrying $+^4$ as opposed to one containing $+^3$. All flies were isogenic for chromosomes I, II and III derived from a highly inbred strain. In each of four populations followed, $+^4$ increased significantly in frequency at the expense of $+^3$ regardless of the initial frequencies of the two chromosomes or the presence of an additional fourth chromosome carrying the recessives *ci* and ey^R. When populations were maintained for fifteen months $+^4$ rose to approximately 90 percent with no indication that an equilibrium had been attained.

3. Due to the apparent lack of adaptive heterosis indicated by mathematical analyses of the results, it appears likely that $+^3$ would have been eliminated in time.

4. Findings in two additional experiments demonstrated that each of the wild type alleles was vastly superior to the *ci* allele present in the *ci* ey^R chromosome. Within a few months each normal allele increased to over 90 percent from original values of 30 percent.

5. If the difference in adaptive values between $+^3$ and $+^4$ determined under laboratory conditions is similar to that encountered in nature, rarity of ci^+ isoalleles of the $+^3$ type would be expected. It is somewhat surprising, however, that "normal" alleles should differ so greatly in fitness under any circumstances. Further investigations are proposed to secure additional information regarding this point.

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LITERATURE CITED

CHETVERIKOV, S. S., 1926 On certain features of the evolutionary process from the viewpoint of modern genetics. J. Exp. Biol. (Russian) **2**: 3–54. (Quoted in DOBZHANSKY, TH., 1951 Genetics and the Origin of Species. Columbia University Press, New York.)

CROW, J. F., and N. E. MORTON, 1955 Measurement of gene frequency drift in small populations. Evolution **9**: 202–214.

DOBZHANSKY, TH., 1950 Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. Genetics **35**: 288–302.

DOBZHANSKY, TH., and H. LEVENE, 1951 Development of heterosis through natural selection in experimental populations of *Drosophila pseudoobscura*. Am. Naturalist **85**: 247–264.

DOBZHANSKY, TH., and B. SPASSKY, 1953 Concealed variability in two sympatric species of Drosophila. Genetics **38**: 471–484.

1954 A comparison of the concealed variability in *Drosophila prosaltans* with that in other species. Genetics **39**: 472–487.

LEFEVRE, G., JR., 1955 Mutation isoalleles at the yellow locus in *Drosophila melanogaster*. Genetics **40**: 374–387.

L'HERITIER, PH., and G. TEISSIER, 1934 Une experience de selection naturelle. Courbe d'elimination du gene "Bar" dans une population de Drosophiles en equilibre. Compt. rend. soc. biol. **117**: 1049–1051.

1937 Elimination des formes mutantes dans les populations de Drosophiles. Cas des Drosophiles "Bar." Compt. rend. soc. biol. **124**: 880–882.

MERRELL, D. J., and J. C. UNDERHILL, 1956 Competition between mutants in experimental populations of *Drosophila melanogaster*. Genetics **41**: 469–485.

MULLER, H. J., 1935 On the incomplete dominance of the normal allelomorphs of white in Drosophila. J. Genet. **30**: 407–414.

SLATIS, H. M., 1955 Current status of information on the induction of mutations by irradiation. Science **121**: 817–821.

SPENCER, W. P., 1938 Multiple alleles at the bobbed locus in populations of *Drosophila hydei*. Genetics **23**: 170.

1944 Isoalleles at the bobbed locus in *Drosophila hydei* populations. Genetics **21**: 520–536.

STERN, C., and E. W. SCHAEFFER, 1943 On wild type isoalleles in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **29**: 361–367.

TIMOFEEFF-RESSOVSKY, N. W., 1932 Verschiedenheit der "normalen" Allele der white-Serie aus zwei geographisch getrennten Populationen von *Drosophila melanogaster*. Biol. Zentr. **52**: 468–476.

WALLACE, B., J. C. KING, C. W. MADDEN, B. KAUFMANN, and E. C. MCGUNNIGLE, 1953 An analysis of variability arising through recombination. Genetics **38**: 272–307.

WRIGHT, S., and TH. DOBZHANSKY, 1946 Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics **31**: 125–156.

THE COMPARATIVE EFFECTS OF HARD AND SOFT IONIZING RADIATIONS ON MALE AND FEMALE RING X DROSOPHILA IN AIR AND NITROGEN[1]

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EARLY in the course of irradiation experiments on *Drosophila melanogaster* it was discovered that in contrast to the results for irradiated males, translocations are almost never recovered in the progeny of irradiated females (PATTERSON and MULLER 1930; SHAPIRO and NEUHAUS 1933; OLIVER 1935; GLASS 1940, 1955a). GLASS (1955a) calculated that the loss (due to aneuploid segregation) of translocations induced in mature oöcytes is three fourths for chromosome exchanges, and fifteen sixteenths for chromatid exchanges. The actual differences greatly exceed the expectancies allowing for this loss. GLASS' data show that the rate for males is a minimum of 19 times as great as that for females given the same dose, and probably much greater. GLASS also observed (1940, 1955a) that the frequency of inversions recovered from the progeny of irradiated females is as much as one fourth to one third that for males. There are several possible explanations for these facts. The observed differences could be due to a sex difference in the rate of breakage of the chromosomes, or to a sex difference in the probability of recombination of broken chromosomes, or both.

GLASS (1955a) has used combined infrared and X-ray treatment of females in an attempt to distinguish between these alternatives with respect to translocations. Some increase was observed in the frequency of translocations when infrared was used in conjunction with X-rays. Together with the relatively smaller depression in females in the frequency of inversions than in the frequency of translocations, this result suggests that the difference in the frequency of aberrations in the two sexes is due to a difference in the probability of recombination. GLASS has also measured the frequency of Minute mutations, some of which are presumably deficiencies, induced in both male and female Drosophila by X-rays (GLASS 1955b). Adult females yielded the same percentage of Minutes as adult males, and any breaks involved in such mutations must, therefore, be as frequent in mature oocytes as in spermatozoa. FABERGÉ (1952) used a more direct, but somewhat less certain approach. He measured the frequency of chromosome breakage in female Drosophila, using the ring X technique first applied by BAUER (1942) to males. Because recombination of the broken ends of different chromosomes is not involved in most induced ring losses, ring loss may be used as a relative

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measure of chromosome breakage. FABERGÉ found that the administration of 2000r of X-rays to X^{e2}/Muller-5 females caused a drop in the ratio of ring X to rod X offspring from 0.87 to 0.73 among the female progeny, and from 1.15 to 0.93 among the male progeny. Recalculation, according to the method of FABERGÉ, of BAUER's figures for the control X^{e2} males and for X^{e2} males given 2000r of X-rays yields figures of 0.97 and 0.71, respectively. Despite the fact that FABERGÉ's figures for female and male progeny of irradiated females showed depressions of 0.14 and 0.22, respectively, while BAUER's data for males yield a depression of 0.28, FABERGÉ remarked that "it can probably be concluded that X-rays produce primary chromosome breaks at about the same rate in the two sexes." KING, DARROW, and KAYE (1956) have performed experiments similar to FABERGÉ's. They gave 2000r of Co^{60} γ-rays to X^{e2}/Mu-5 females and scored both the ratio of ring X to rod X offspring and the rate of production of XO males in daily broods for 35 days. In contrast to FABERGÉ's results, they found an appreciable rate of ring loss only in the first few days after treatment. Recalculation of the data for their one day sample from irradiated 3–4 day old females, the only one which is comparable to my own experiments, gives ring loss figures of 2.2 percent for the female offspring and 1.4 percent for the male offspring. Unfortunately the sample size was not large enough to make these differences statistically significant.

Another phenomenon which may be explained by a reduction in breakage, a reduction in recombination, or both, is the effect of anoxia on the rate of induction of chromosome aberrations by X-rays. That the oxygen tension at the time of irradiation has an effect on the aberration frequency has been known for some time. THODAY and READ (1947, 1949), measuring anaphase chromosome aberrations in *Vicia faba* root tips, found that an increased oxygen tension increased the frequency of aberrations, and that anoxia decreased the frequency of aberrations induced by X-rays. GILES, RILEY, and BEATTY (GILES and RILEY 1949, 1950; GILES and BEATTY 1950; GILES 1952; GILES, BEATTY and RILEY 1952; RILEY, GILES and BEATTY 1951, 1952) have shown that oxygen has a similar effect on the X-ray-induced frequency of both chromosome and chromatid aberrations in Tradescantia, while SCHWARTZ (1952) has demonstrated the oxygen effect in maize. HOLLAENDER, BAKER and ANDERSON (1951) BAKER and EDINGTON (1952) have demonstrated that X-ray-induced translocations in *Drosophila virilis* show an oxygen dependence similar to that demonstrated in Vicia, Tradescantia, and Zea. BAKER and VON HALLE (1953), studying X-ray-induced dominant lethals in Drosophila sperms, found that anoxia reduced the rate of this (presumed) sort of chromosome aberration also. LÜNING (1954), in a similar study of both males and females, confirmed the finding of BAKER and VON HALLE and found that anoxia also reduces the frequency of X-ray induced dominant lethals in the female. In contrast, BAKER and VON HALLE (1954) found that the rate of X-ray-induced losses of ring X (X^{e1}) chromosomes was not markedly affected by the oxygen tension at the time of irradiation; and they expressed the view that their work demonstrates that the oxygen effect is mainly on recombi-

nation, rather than on breakage. A number of studies have demonstrated that the oxygen effect is not constant with respect to the type and energy of the radiation employed. THODAY and READ (1949) showed that the oxygen effect on Vicia aberrations was much smaller when α particles were used than when X-rays were used. GILES, BEATTY and RILEY (1952) demonstrated that air/nitrogen ratios for chromatid aberrations induced by neutrons were intermediate between those for X-rays and those for α particles. SWANSON (1955a,b) demonstrated that in Tradescantia the air/nitrogen ratios depend on the wave length of the radiation used. In particular, he found that isochromatid deletions induced in nitrogen show no dependence upon the hardness of the radiation used to induce them, whereas they are proportional to the ion density of the photon path when induced in air. GILES and his co-workers expressed the view, on the basis of their work, that the oxygen effect is mainly on breakage, in large part because posttreatment of irradiated buds of Tradescantia with a high oxygen concentration had no effect on the aberration rate (GILES and RILEY 1950). SWANSON, on the other hand, feels that the Tradescantia data can only be explained by assuming that the oxygen effect is on both recombination and breakage. LÜNING (1954) feels that this view also best explains his Drosophila data. Thus BAKER and VON HALLE's study on the loss of ring X's in Drosophila (1954) is the most important piece of evidence supporting the "restitution only" hypothesis.

BAUER (1942), in his study of X-ray-induced loss of ring X chromosomes in male Drosophila, found that the percentage of loss of X^{c2} chromosomes at 2000r in air was 7.7 percent. BAKER and VON HALLE (1954) found that the rate of loss of X^{c1}, y at 2000r in air was 8.8 percent while for the same dose in nitrogen the rate was 7.9 percent. One difference between the two experiments, other than the difference in the stocks used, was in the wave length of the X-rays used. BAUER used 115 kVp X-rays, filtered by 1 mm of Al, while BAKER and VON HALLE used 250 kVp X-rays, filtered by 2 mm of Al. Thus it seems possible that for ring X loss in Drosophila, which may be compared to isochromatid deletions in Tradescantia, there is a wave length effect, similar to that found by SWANSON (1955a,b).

In view of the facts cited above, two questions arise. The first is whether the breakage rate is the same in Drosophila females as in males. The second is whether or not there is an influence of wave length on the air/nitrogen ratio.

MATERIALS AND METHODS

Biological

Two ring X chromosomes were used in these experiments; X^{c1}, y and X^{c2}. Both are fully described elsewhere (SCHULTZ and CATCHESIDE 1937). Ring chromosomes have the peculiarity that a single break might be followed by any one of as many as three different types of refusion of the broken ends, only one of which leads to viable daughter chromosomes. The relative probabilities of the various events which follow a break in a ring X chromosome are not known, and hence it is not possible to measure the absolute break frequency by measuring

ring loss. It has been shown, however, that ring loss is a *relative* measure of break frequency (BAUER 1942).

The ring X chromosome stocks used in the following experiments were all obtained from single males immediately before beginning a given series of experiments. Thus all of the ring chromosomes used in a single series of experiments were presumably isogenic. After a stock was obtained, the chromosome was checked cytologically in aceto-orcein smears of third instar larval neuroblasts.

In all of the experiments, ring X males were mated immediately after treatment to *y ct*^{6} *ras*^{2} *f* virgin females. All of the treated females in the comparable series were heterozygous for one of the ring chromosomes and the Muller-5 chromosome. The Muller-5 chromosome [*Ins(1) sc*^{S1} *sc*^{8} *InS, B w*^{a}] is described by SPENCER and STERN (1948). It was used in these experiments because it had been used for the same purpose by FABERGÉ (1952). Virgin females were treated and mated immediately thereafter to *y ct*^{6} *ras*^{2} *f* males.

All of the flies used in both series of experiments (treated males; treated females) were between one and four days old. All matings were made in one half pint milk bottles containing a sugar, agar, cornmeal and brewers' yeast medium. Twenty pairs of flies were used per bottle. The parents were allowed to remain in the bottles for five days, after which they were discarded, except in one experiment wherein irradiated females were remated and then allowed to lay eggs in new bottles for an additional five days. The bottles were kept at 25° ± 1°C for the duration of the experiment. When the F_1 flies began to eclose, they were counted, usually every other day, up to and including the nineteenth day after the mating of the P_1. Because usually only a few flies eclosed on the last few days, it is assumed that virtually all of the F_1, but none of the F_2, were counted. In a few bottles from experiments where F_2 flies could be distinguished from the F_1, it was observed that the F_2 flies did not begin to eclose until the twenty-first day after mating.

The problem raised by the heterogeneity between replications of the same experiment is one that is very troublesome, and one that has often been ignored by Drosophila workers. In the present experiments it proved impossible to obtain control series, for example, that in different replications would yield exactly the same ring X/rod X ratios, or even ratios that were not significantly different, in a statistical sense. The same variability, of course, affected the series of individuals treated in air or in nitrogen. When, however, an experiment included as many as four classes per series, and three series (control, X-rayed in air, X-rayed in nitrogen), as here, the replication of each experiment three times results in a three-dimensional matrix of 36 cells, among which, on the basis of normal probability, about two would be expected to show chi-square deviations significant at the five percent level of probability.

It may happen, on the other hand, that although the series show significant heterogeneity from unestablished causes, nevertheless the differences of each series from its own control and the differences between experimental series within the same replication are always, in successive replications, in the same direction

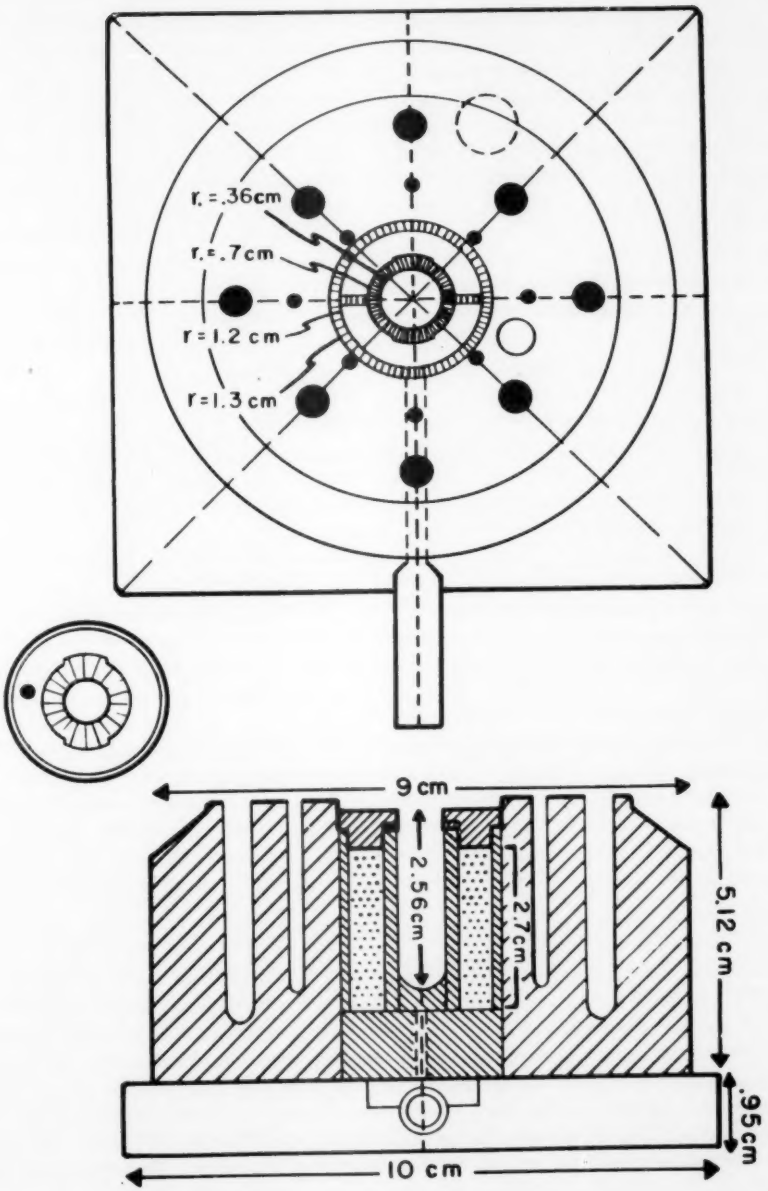
and of approximately the same magnitude. In that case, as here, it seems legitimate to pool the replications, since the effect of doing so is to randomize the uncontrolled variability and to establish on a significant level any systematic differences. With proper precautions, this procedure has been followed.

Physical

Two sources of ionizing radiations were used. Soft X-rays were generated by a Westinghouse industrial machine operated at 60 kVp. The only filtration was that inherent in the beryllium tube window (equivalent to 0.6 mm of Al). The target distance was 16 cm. The tube was always operated at 10 ma anode current. The dose rate varied between 200 and 215r per minute for various experiments. All dose measurements were made with a Victoreen condenser dosimeter with a 100r bakelite chamber. This instrument was calibrated by the National Bureau of Standards during the course of the experiments and was found to be correct within three percent at the anode voltage used. The half-value layer for the X-ray tube under the above operating conditions was determined and is 0.82 mm of Al. For all X-ray experiments exposures were made in a gas-tight lucite chamber with a 6.3 mm top, through which the X-rays passed. This chamber was equipped with a port through which the Victoreen ionization chamber could be introduced to the point to be occupied by the flies. The flies were enclosed in perforated gelatin capsules during treatment. For air exposures the chamber was left open to the ambient atmosphere. For nitrogen exposures, commercial compressed nitrogen (99.9 percent pure) was passed through the chamber at a rate of about ten liters per minute for five minutes before irradiation; during irradiation the flow was reduced to about five liters per minute. Both males and females were irradiated simultaneously, and the nitrogen exposures were always made immediately after the air exposures.

The γ-rays used were those of radium, having an energy spectrum of from 0.2 to 2.2 meV. For the γ exposures a special combination jig and chamber was constructed of lucite (see Figure 1). As will be seen from the figure, the fly chamber is of an annular shape, surrounding a central well for a radium source. The annulus was divided into two halves by perforated lucite partitions. The unit consisted of three subunits: a base and fly chamber assembly, a cap for the fly chamber which locked into place by means of bayonet lugs, and a doughnut-shaped jig block with wells for 16 radium needles. The jig block fitted snugly over the fly chamber and was held in position by a dowel pin. The cap for the fly cham-

FIGURE 1.—Plan of the lucite jig and chamber assembly used in the γ-irradiations. A top view, cap removed, is shown on the left, with the cap in bottom view at the center. Outer radium wells black, fly chamber and central radium well clear. Dowel to hold jig in position shown as dashed circle in top view. A sectional side view with the cap in place is shown on the right. The outer eight holes have a diameter of 0.5 cm and a depth of 3.85 cm. The inner eight holes have a diameter of 0.1 cm and a depth of 3.4 cm. The holes are spaced on the following radii from the center: inner four 0.1 cm holes, 1.5 cm; outer four 0.1 cm holes, 2.0 cm; inner four 0.5 holes, 2.5 cm; outer four 0.5 holes, 3.0 cm.


r = .36cm
r = .7cm
r = 1.2 cm
r = 1.3 cm
9 cm
2.56cm
2.7cm
5.12 cm
.95cm
10 cm

ber was provided with a cork gasket which prevented gas leakage. Entering gas passed to one side of the fly chamber from the inlet tube. After passing to the other side through the perforated dividers, it was allowed to escape through a port in the cap. Cotton plugs (top and bottom) prevented the escape of flies through the gas inlet or outlet and ensured that the fly chamber was packed tightly with flies. The jig block's 16 radium wells accommodated standard therapeutic radium needles of two types. In the two inner circles of four, the needles held ten millicuries each and were screened by one half of a millimeter of platinum. The two outer circles of four also held ten millicurie needles, but these needles had one millimeter silver screens (equivalent to 0.6 mm of platinum) in addition to the one half millimeter platinum screens. The central source was a one millimeter thick aluminum shell, loaded with three radium needles, each shielded by one half millimeter of platinum, which totaled 150 millicuries. The depth of all radium source wells and of the fly chamber was such that the isodose lines were parallel to the walls of the fly chamber for its entire length.

In use, the chamber was loaded with flies and placed in a lead brick safe, and, in the case of nitrogen exposures, flushed with nitrogen at a rate of two liters per minute for five minutes, after which the rate was reduced to one liter per minute. The outer jig block (already loaded with the 16 radium needles) and the central source were then removed from their protective containers and placed in their irradiating positions as quickly as possible.

During irradiation the dose rate was monitored with a Bomke integrating dosimeter, the ionization chamber of which was placed in the lead brick safe at a point 10.8 centimeters from the center of the fly chamber. The dose rate at this point was 0.25r per minute. Measurements were also made at the center radium well with the central source removed. The dose rate at this point was 3.2r per minute. All four exposures were made for 27.4 minutes, the time calculated from preliminary measurements to give a dose of 1000r. A dose of 1000r was used because it was found necessary to limit the time of the nitrogen exposures. The physical arrangement of the radium sources was designed to give as uniform a dose over the entire fly chamber as possible. Later, and more accurate, calculations showed that the dose received at the inner edge of the fly chamber was 1450r, while the dose at the outer edge of the fly chamber was 650r. The mean dose received by the flies was 1050r.

EXPERIMENTAL RESULTS

X-ray experiments with X^{c2}

The results of the experiments with X^{c2} are given in Table 1. Each experiment was repeated three times; in each case the males and the females were irradiated simultaneously. In one experiment the control series was lost, due to circumstances beyond control. In spite of the fact that one of the classes (the w^a *B* male class of the control of the treated-female experiments) differed significantly from the expected in a chi-square test of the replications of each part of the experiments, the counts were pooled, for the reasons given above. The correction of

TABLE 1

Ring loss data calculated for the F_1 progeny of X-rayed X^{c2} Muller-5 females and X^{c2} males

Sex of treated parent	Treatment	$N_{tot.}$	N_{ring}	Percent rings	Corr.* percent rings	Diff. from control
			Female progeny			
Female	None (control)	9,853	4,983	50.6	50.0±0.5	
	2000r of X-rays in air	11,501	5,885	51.2	50.6±0.5	+0.6±0.7
	2000r of X-rays in nitrogen	10,736	5,345	49.8	49.2±0.5	−0.8±0.7
			Male progeny			
	None (control)	7,958	4,688	58.9	50.0±0.6	
	2000r of X-rays in air	9,022	4,957	54.9	46.6±0.5	−3.4±0.8
	2000r of X-rays in nitrogen	9,141	5,071	55.5	47.1±0.5	−2.9±0.8
Male	None (control)	14,548	8,557	58.8	50.0±0.4	
	2000r of X-rays in air	15,815	7,860	49.7	42.3±0.4	−7.8±0.6
	2000r of X-rays in nitrogen	20,367	10,406	51.1	43.4±0.4	−6.6±0.6

* The correction factor, based on the control, $\frac{N_{tot.}}{2\,N_{ring}}$, is that given by BAUER (1942).

BAUER (1942), based on the control series for each separate experiment, was made in all of the X-ray experiments so that the data could be directly compared. Air/nitrogen ratios were calculated by dividing the percentage difference from the control for the air-treated sample by the percentage difference from the control for the nitrogen-treated sample. The experimental results will be discussed in conjunction with the X^{c1}, *y* data which follow.

X-ray experiments with X^{c1}, y

Because it was suspected that the X^{c2} ring chromosome might respond to radiation in a different fashion than the X^{c1}, *y* ring, the above experiments were repeated using X^{c1}, *y*. The results of these experiments are presented in Table 2. The table represents the pooled data from two replications of the experiment. In each case the P_1 males and the P_1 females were irradiated simultaneously. A chi-square analysis of the differences between the two replications shows that they were significantly different in the frequencies of two classes. Included in the female data are the F_1 from both samples of an experiment in which the P_1 females were

TABLE 2

Ring loss data calculated for the F_1 progeny of X-rayed X^{c1}, y/Muller-5 females and X^{c1}, $y/y^+sc^8.Y$ males

Sex of treated parent	Treatment	$N_{tot.}$	N_{ring}	Percent rings	Corr.* percent rings	Diff. from control
			Female progeny			
Female	None (control)	17,361	8,118	46.8	50.0±0.4	
	2000r of X-rays in air	15,785	6,969	44.1	47.1±0.4	−2.9±0.6
	2000r of X-rays in nitrogen	13,951	6,407	45.9	49.1±0.4	−0.9±0.6
			Male progeny			
	None (control)	14,790	6,806	46.0	50.0±0.4	
	2000r of X-rays in air	12,332	5,492	44.5	48.4±0.4	−1.6±0.6
	2000r of X-rays in nitrogen	11,239	5,203	46.3	50.3±0.5	+0.3±0.6
Male	None (control)	19,793	9,632	48.7	50.0±0.4	
	2000r of X-rays in air	14,575	5,972	41.0	42.1±0.4	−7.9±0.6
	2000r of X-rays in nitrogen	12,044	5,193	43.1	44.3±0.5	−5.7±0.6

* See table 1.

allowed to lay eggs for the first five days and then put in a second set of bottles and allowed to lay eggs for an additional five days. There was no significant difference between the two broods.

Table 3 presents, in simplified form, the X-ray-induced ring loss data for X^{c1}, y and for X^{c2}, for both males and females. Where the ring loss figures are not significantly different from the control values, the word "none" is entered in the "ring loss percent" column. It may be seen that the two ring X chromosomes do not respond to X-irradiation in quite the same manner, at least when the chromosome is irradiated in the female. The X^{c1}, y chromosome also seems to show a greater protection by nitrogen than the X^{c2} chromosome. While the difference between the values in the male series is not quite significant ($P = 0.07$), there is a striking difference in the amount of protective effect when the rings are irradiated in the females.

X-ray experiments with In(1) X^{c1}, y

In order to test the frequency of loss of rod X chromosomes from irradiated females, relative to ring X loss, an experiment was performed in which the *In(1)*

X^{c1}, y chromosome (BENDER, in press) was used instead of X^{c1}, y. Since the two chromosomes presumably have the same genotype (because one was derived from the other), any difference in their X-ray-induced rates of loss would most likely be due to the contrast between the ring form of X^{c1}, y and the rod form of *In(1)* X^{c1}, y. BAUER (1942) has performed a similar experiment with treated males. He found that the rod X was lost at a rate of about 0.5 percent per 1000r greater than the rate of loss of the Y chromosome. Table 4 presents the results of a cross in which *In(1)* X^{c1}, y/Muller-5 treated females were mated to Muller-5 males. There is no significant difference between the control and the irradiated samples, for either the male or the female progeny. It may be safely concluded, then, that all of the ring loss from the female series recorded in Table 3, to the extent that it is real, is due to the ring shape of the X^{c2} and X^{c1}, y chromosomes.

γ-ray experiments with X^{c1}, y

The results of two experiments in which X^{c1}, y/Muller-5 females and X^{c1}, y/y^+ sc^8-Y males were irradiated with γ-rays in both air and nitrogen are given in Table 5. Although there was a significant difference between the results for the two experiments, the F_1 counts were pooled in calculating the data, as explained above. No control series was run because the only information desired

TABLE 3

Ring loss at 2000r of X-rays, measured as percentage difference from respective controls in air and nitrogen

Treated parent	Sex of F_1	Gas treatment	Ring loss percent	Diff. between air and N_2	air / N_2
X^{c2} males	both*	air	7.8±0.4	1.1±0.6	1.17
		nitrogen	6.6±0.4		
X^{c1}, y males	both*	air	7.9±0.4	2.2±0.7	1.39
		nitrogen	5.7±0.5		
X^{c2} females	female	air	none	none	...
		nitrogen	none		
	male	air	3.4±0.8	0.5±0.8	...
		nitrogen	2.9±0.8		
X^{c1}, y female	female	air	2.9±0.6	2.0±0.6	3.22
		nitrogen	none		
	male	air	1.6±0.6	1.9±0.7	∞
		nitrogen	none		

* See text.

TABLE 4

Losses of the In(1) X^{c1}, y *chromosome calculated for the progeny of X-rayed* In(1) X^{c1}, y/*Muller-5* P_1 *females*

Treatment	$N_{tot.}$	$N_{inv.}$*	Percent inv.*	Corr.† percent inv.*	Diff. from control
		Female progeny			
None (control)	17,451	10,106	57.9	50.0±0.4	
2000r of X-rays in air	10,081	5,780	57.3	49.3±0.5	0.7±0.6
		Male progeny			
None (control)	14,277	8,170	57.2	50.0±0.4	
2000r of X-rays in air	7,978	4,585	57.5	50.3±0.6	+0.3±0.7

* inv. = *In(1)* X^{c1}, *y*.
† See table 1.

TABLE 5

The effect of nitrogen on γ*-ray-induced* X^{c1}, y *loss in males and females*

Sex of parent	Sex of F_1	Gas treatment	$N_{tot.}$	N_{ring}	Percent rings	Diff. between air and N_2
Female	Female	air	17,942	8,383	46.7±0.4	+0.5±0.6
		nitrogen	18,487	8,525	46.1±0.4	
	Male	air	14,510	6,951	47.9±0.4	+1.9±0.6
		nitrogen	14,969	6,883	46.0±0.4	
Male	Both*	air	13,074	6,511	49.8±0.4	+1.3±0.6
		nitrogen	11,907	5,777	48.5±0.5	

* See text.

was to know whether or not nitrogen protects against ring loss when high energy radiation is used. It may be seen that there is no protective effect.

DISCUSSION

Male and female breakage rates

While it is impossible to make a direct comparison between the present experiment with X^{c2} females, and FABERGÉ's (1952) data on females with the same ring X (because of the brevity of FABERGÉ's report), it is obvious that the results are not in agreement with his (see Table 1). He found a depression in the frequency of offspring carrying the ring X in both the male and the female progeny

of X^{c2} females given 2000r of X-rays. My own experiments do not demonstrate any significant depression in the latter (Table 1, top), and, while there is a significant depression in the frequency of X^{c2} males among the male progeny, it is quite small. FABERGÉ concluded that the rate of ring loss in the progeny of treated females was the same in his own experiments as the rate found by BAUER (1942) for X^{c2} males. The present repetition of the experiment makes the generality of FABERGÉ's conclusion extremely doubtful, because the rate of ring loss in the male progeny of treated X^{c2} females (3.4 percent) is only half of the rate of ring loss in the offspring of treated males (7.8 percent). In calculating the relationship between the rates of loss for males and for females, it is necessary to correct the rate for males, because BAUER found that rod X chromosomes were lost at a rate of about 0.5 percent per 1000r greater than the rate for Y chromosomes, while my own experiments with *In(1)* X^{c1}, *y* heterozygotes (Table 4) indicate that ring X's in females are lost only because of their ring shape. At a dose of 2000r, then, about one percent out of the total 7.8 percent rate for males is due to some cause that does not exist in females. Even when this correction is made, however, the rate of ring loss from the progeny of irradiated X^{c2} females in the present experiments is at most only 59 percent of the rate of loss in males. KING, DARROW and KAYE (1956) concluded that the difference between their results and those of FABERGÉ was due to the fact that FABERGÉ did not attempt to differentiate between the rate of ring loss in more or less mature female germ cells. No such difference, of course, could be the reason for the difference between the present results and those of FABERGÉ.

The X-ray experiments with X^{c1}, *y* give approximately the same results as those with X^{c2}, except that there is a significant loss of the ring chromosome in *both* sexes of offspring from irradiated X^{c1}, *y* females (Table 2). In fact, for this ring chromosome the greatest rate of loss occurs in the *female* progeny. This difference in the response of the two chromosomes when they are irradiated in the oöcyte, if real, is quite interesting. It may only be explained by assuming that the two chromosomes behave differently when involved in an anaphase bridge, due either to sister-strand fusion or torsional refusion, after a break. If, in the oöcyte, X^{c2} anaphase bridges almost always break, one would expect to find only a small reduction in the number of female offspring which carried rings, since most of the resulting deficient chromosomes, although lethal in an XY zygote, would be "covered" by the paternal X chromosome in XX zygotes. This would be particularly true if breakage of the X^{c2} anaphase bridge always occurred at a point close to the centromere, such as the point of attachment of the *bb* end of the euchromatin to the proximal heterochromatin. If X^{c1}, *y* bridges seldom break, one would anticipate that the ring X class of both sexes of offspring would be reduced by the same amount through the production of nullo X eggs. If both events occurred, the frequency of loss of the ring chromosome from the progeny of irradiated ring X females would be somewhat lower in the female progeny than in the male progeny.

The maximum estimate for the frequency of loss of ring X chromosomes in

oöcytes irradiated in air is roughly one half of the frequency of loss of ring X chromosomes from sperms irradiated in air (see above). Not only is this result in conflict with the results of FABERGÉ, but it does not lead to the same conclusion as that reached by GLASS (1955b), namely, that X-ray-induced breaks are equally as frequent in oöcytes as in mature sperms. GLASS found that Minute mutations, induced by X-rays, and presumed to be "single-hit" deletions, were equally frequent in the progeny of males and females, at least at dosages up to 4000r.

This apparent disagreement may be resolved in several ways. If one assumes that breakage does occur with the same frequency in males and females, it is then necessary to assume that the events which follow breakage are different in the two sexes. Ring chromosome loss following breakage can occur in two different ways; either through sister-strand fusion or through torsional refusion. The low frequency of loss of ring chromosomes in the progeny of irradiated females might consequently be explained by the absence of (or a reduction in) one or the other type of recombination in females.

Even if one assumes that breakage occurs less frequently in irradiated oöcytes than in irradiated sperms, it is still necessary to assume that the events which follow breakage are different in the two sexes. To explain the finding of GLASS, it becomes necessary to assume that most Minutes are actually "two-hit" events, as a good many unquestionably are. The dosage curve published by GLASS for Minutes fits this hypothesis very well, for the linear portion up to 2000r is not significantly different from the lower portion of an exponential curve. Only a logical extrapolation of the facts already observed by GLASS (1940, 1955a, 1955b) is necessary to explain the disagreement between the results for Minutes and for ring X loss. A striking fact, pointed out by GLASS, is that "two-hit" aberrations induced in oöcytes show the greatest reduction in rate below the rate for sperms when the breaks are far apart, as in translocations. The rate in oöcytes is much less reduced below the rate for sperms when the breaks are nearer, as in the case of inversions, and are even less reduced in the case of deficiencies. If one extrapolates, one might logically expect that there would be an interbreak distance where the frequency of small deletions and inversions would be equal in males and in females, even though the frequency of breaks in the two sexes was not the same. Indeed, the small excess of Minute mutations from irradiated females which is noted by GLASS may be explained on this hypothesis.

The oxygen effect

As pointed out in the introduction, BAKER and VON HALLE (1954) were not able to demonstrate any effect of oxygen on the loss of X^{c1}, *y* from the progeny of males treated with low or moderate doses of hard X-rays. It may be seen from Tables 1 and 2, however, that the present experiments, which utilized relatively soft X-rays, reveal a significant difference between the results for X^{c1}, *y* males treated in air and those treated in nitrogen (Table 2), and a suggestive difference for X^{c2} males ($P = 0.07$, see Table 1). This difference between the present results

and those of BAKER and VON HALLE might be expected, however, in the light of the work of SWANSON (1955a,b) on the wave length dependence of isochromatid deletions in Tradescantia, for SWANSON found that the air/nitrogen ratio for these aberrations decreases with decreasing ion density (increasing energy) of the radiation employed. A similar relationship is observed when the results of BAKER and VON HALLE are compared with my own for X^{c1}, y males. While my own data yield an air/nitrogen ratio of 1.39 for the progeny of X^{c1}, y males given 2000r of unfiltered 60 kVp X-rays (Table 3), BAKER and VON HALLE found a ratio of only 1.09 for the progeny of X^{c1}, y males given 2000r of 250 kVp X-rays filtered by 2 mm of Al. The γ-ray experiments were performed to check this relationship. Reference to Table 5 will show that there is no significant difference between the results from X^{c1}, y males irradiated with γ-rays in air and those irradiated in nitrogen. The differences are, in fact, consistently in the direction of a somewhat greater effect in nitrogen than in air.

The X-ray experiments with X^{c2} females (Table 1) did not demonstrate any significant oxygen effect. An oxygen effect is clearly shown, however, in the experiments with X^{c1}, y females (Table 2). As to the consistency of the oxygen effect in the progeny of X-rayed males and females, it is clear from Table 3 that while there is no significant percentage difference between the results for X^{c1}, y females and males, the females show a greater *relative* protection (air/nitrogen ratio) than do the males.

When γ-rays were used to irradiate X^{c1}, y females, the rate of ring loss from the progeny of females irradiated in air was lower than the rate of ring loss from the progeny of females irradiated in nitrogen. This fact confirms the effect of wave length on the air/nitrogen ratio for ring X chromosome loss already noted for males.

BAKER and VON HALLE (1954) and BAKER (1955) suggested that the oxygen effect is on restitution alone, and not on the breakage rate. BAKER has argued (1955) that if one assumes that there is a high probability of both normal and torsional restitution of breaks in a ring X chromosome, and if the effect of oxygen is to increase the rate of restitution of both types, the air/nitrogen ratio would be small, as is indeed the case. Yet the assumption that the smallness of the air/nitrogen ratio indicates that the effect of oxygen is *only* to increase the rate of restitution does not seem valid, since the small air/nitrogen ratio could be caused by a number of other factors. The present experiments offer proof that the effect of oxygen on the rate of loss of ring X chromosomes from the progeny of irradiated X^{c1}, y males *cannot* be due to an effect on restitution alone. For since the γ-ray experiments show a definite decrease of the air/nitrogen ratio to unity with increasing hardness of the radiation used, it seems certain that the effect of anoxia is on a class of damage caused by the soft X-rays, but not by the hard γ-rays. There is good reason to believe that this class consists of "potential" breaks, which become actual breaks much more often in the presence of oxygen than in its absence.

THODAY (1953) has postulated that at least some breaks originate as "potential

breaks," which either may or may not become actual breaks, depending on the physiological conditions in the cell at the time. LÜNING (1954) and SWANSON (1955a,b) have extended this concept to include a whole "spectrum" of chromosome damage. They postulated that oxygen is effective in converting potential breaks into actual breaks. They further postulated, on the basis of the Tradescantia data, that oxygen must also have an effect on the rate of rejoining of breaks. The present experiments not only accord with this hypothesis, but they offer some measure of confirmation for it. Another possible explanation of both the present data and those of SWANSON, namely, that breaks induced by γ-rays differ qualitatively in their ability to rejoin in air from those induced by X-rays, seems much less likely, although the present experiments do not exclude that possibility completely.

SUMMARY

The rate of loss of ring X chromosomes in the progeny of X-irradiated males and females has been studied, using both the X^{c1}, y chromosome and the X^{c2} chromosome. It is shown that breakage, measured as ring loss, is less frequent in X-rayed oöcytes than in X-rayed sperms. This finding and that of GLASS (1955b), that X-ray-induced Minute mutations in oöcytes are as frequent as those induced in males, are explained by means of an hypothesis containing two assumptions. The first is that Minute mutations are composed of a substantial proportion of "two-hit" events; the second, also postulated by GLASS, is that recombination of breaks which are very close together is more likely in oöcytes than in sperms, while recombination of breaks which are more distant is more likely in sperms than in oöcytes.

A further study has been made of the rates of ring loss in males and in females, in both air and nitrogen, induced by soft X-rays and by hard γ-rays. When X-rays were used, these studies showed a small, but quite significant, effect of oxygen on ring loss in X^{c1}, y males, and a larger oxygen effect on ring loss in X^{c1}, y females. When γ-rays were used, however, there was no demonstrable effect of oxygen on the frequency of ring loss in either sex. This result is interpreted in the light of the hypothesis of a "spectrum of chromosome damage" postulated by LÜNING (1954) and by SWANSON (1955a,b).

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LITERATURE CITED

BAKER, W. K., 1955 The oxygen effect and the mutation process. *Mutation.* Brookhaven Symposia Biol. **8**: 191–200.

BAKER, W. K., and C. W. EDINGTON, 1952 The induction of translocations and recessive lethals in Drosophila under various oxygen concentrations. Genetics **37**: 665–667.

BAKER, W. K., and E. S. VON HALLE, 1953 The basis of the oxygen effect on X-irradiated Drosophila sperm. Proc. Natl. Acad. Sci. U.S. **39**: 152–161.

1954 Evidence on the mechanism of the oxygen effect by use of a ring chromosome. J. Cell. Comp. Physiol. (Suppl. 2) **45**: 299–307.

BAUER, H., 1942 Röntgenauslosung von Chromosomenmutationen bei *Drosophila melanogaster*. II. Die Haufigkeit des primären Brüchereignisses nach Untersuchungen an Ring-X-Chromosom. Chromosoma **2**: 407–458.

FABERGÉ, A. C., 1952 Loss of ring chromosomes produced by X-rays in female Drosophila. (Abstr.) Rec. Genetics Soc. Am. **21**: 23.

GILES, N. H., JR., 1952 Recent evidence on the mechanism of chromosome aberration production by ionizing radiations. pp. 267–284. *Symposium on Radiobiology (Oberlin, 1950)*. Edited by J. J. NICKSON. John Wiley & Sons, Inc. New York.

GILES, N. H., JR., and A. V. BEATTY, 1950 The effect of X-irradiation in oxygen and in hydrogen at normal and positive pressures on chromosome aberration frequency in Tradescantia microspores. Science **112**: 643–645.

GILES, N. H., JR., and H. P. RILEY, 1949 The effect of oxygen on the frequency of X-ray induced chromosomal rearrangements in Tradescantia microspores. Proc. Natl. Acad. Sci. U.S. **35**: 640–646.

1950 Studies on the mechanism of the oxygen effect on the radio-sensitivity of Tradescantia chromosomes. Proc. Natl. Acad. Sci. U.S. **36**: 337–344.

GILES, N. H., JR., A. V. BEATTY, and H. P. RILEY, 1952 The effect of oxygen on the production by fast neutrons of chromosomal aberrations in Tradescantia microspores. Genetics **37**: 641–649.

GLASS, H. B., 1940 Differential susceptibility of the sexes of Drosophila to the effects of X-rays in producing chromosome aberrations. (Abstr.) Rec. Genetics Soc. Am. **8**: 117.

1955a A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. I. Translocations and Inversions. Genetics **40**: 259–267.

1955b A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. II. Deficiencies and Minutes. Genetics **40**: 281–296.

HOLLAENDER, A., W. K. BAKER, and E. H. ANDERSON, 1951 Effect of oxygen tension and certain chemicals on the X-ray sensitivity of mutation production and survival. Cold Spring Harbor Symposia Quant. Biol. **16**: 315–326.

KING, R. C., and JEAN B. DARROW, and NANCY WEBER KAYE, 1956 Studies on different classes of mutations induced by radiation of *Drosophila melanogaster* females. Genetics **41**: 890–900.

LÜNING, K. G., 1954 The effect of oxygen on irradiated males and females of Drosophila. Hereditas **40**: 295–312.

OLIVER, C. P., 1935 Genetic effects induced in the sexes. Am. Naturalist **69**: 74.

PATTERSON, J. T., and H. J. MULLER, 1930 Are "progressive" mutations produced by X-rays. Genetics **15**: 495–578.

RILEY, H. P., N. H. GILES, JR., and A. V. BEATTY, 1951 The oxygen effect on X-ray-induced chromatid aberrations in Tradescantia microspores. (Abstr.) Rec. Genetics Soc. Am. **20**: 120.

1952 The effect of oxygen on the induction of chromatid aberrations in *Tradescantia* microspores by X-irradiation. Am. J. Botany **39**: 592–597.

SCHULTZ, J., and D. G. CATCHESIDE, 1937 The nature of closed X-chromosomes in *Drosophila melanogaster*. J. Genet. **35**: 315–320.

SCHWARTZ, D., 1952 The effect of oxygen concentration on X-ray-induced chromosome breakage in maize. Proc. Natl. Acad. Sci. U.S. **38**: 490–494.

SHAPIRO, N., and M. NEUHAUS, 1933 A comparative study of the mutation process in the female and male of *Drosophila melanogaster*. Biol. Zh. Mosc. **2**: 425–445.

SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r-dose/mutation frequency relation in *Drosophila* at low dosage. Genetics **33**: 43–74.

SWANSON, C. P., 1955a Relative effects of qualitatively different ionizing radiations on the production of chromatid aberrations in air and in nitrogen. Genetics **40**: 193–203.

1955b The oxygen effect and chromosome breakage. J. Cell. Comp. Physiol. **45**: 285–298.

THODAY, J. M., 1953 Sister-union isolocus breaks in irradiated *Vicia faba*: The target theory and physiological variation. Suppl. Heredity **6**: 299–309.

THODAY, J. M., and J. READ, 1947 The effect of oxygen on the frequency of chromosomal aberrations produced by X-rays. Nature **160**: 608.

THODAY, J. M., and J. READ, 1949 Effect of oxygen on the frequency of chromosome aberrations produced by alpha-rays. Nature **163**: 133–134.

THE OCCURRENCE AND GENETIC BEHAVIOR OF DUPLICATE LOWER INCISORS IN THE MOUSE*

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A new trait in the mouse, involving the appearance of extra lower incisor teeth, seems worthy of note because of its morphological interest and its unusual genetic behavior. The trait, which may be designated as *di* (duplicate incisors), is characterized by the appearance on one or both sides of a supernumerary and somewhat aberrant tooth located close to the mandibular symphysis and directly behind or slightly medial to the corresponding normal tooth. The accompanying figures show duplicate teeth as they appear in a living animal and in a cleaned lower jaw. A brief description of the trait, a few circumstances surrounding its origin, and some preliminary breeding records can be reported at this time.

Normal lower incisors erupt at about the eleventh day of postnatal life and the accessories, with less regularity, a few days later, but their rudiments when present can be demonstrated in newborn and prenatal young. Each accessory has its own alveolus, which is so close to that of the normal tooth that the two may seem to emerge from the same socket. The duplicates are usually clavate at first and for a brief period may appear to be as large as, or even larger than the normals. Later, they tend to lose their bulbous tips and become smaller by comparison and more uniform in diameter. A few are closely appressed to the normal teeth with their tips correspondingly beveled, but most of them become deflected and are ultimately broken off or displaced from their sockets. When the latter happens, bone of the alveolar process soon fills in the deficiency making it difficult or impossible to say whether or not an extra tooth has ever been present. But not all duplicates are lost, some of them, or their broken roots, remaining indefinitely. Such persistent remnants often force the normal teeth sufficiently out of alignment to cause serious malocclusion, an eventuality that can be avoided if need be by early extraction of the duplicates. Thus far, aside from indirect effects of malocclusion, possibly somewhat reduced vigor, and a rather high incidence of mammary tumors, no indication has been found of other physical or physiological peculiarities that might be attributed to *di* genes.

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MATERIAL

Origin of the di *trait*

In view of the increased interest in mutagenesis that has been engendered by the "atomic age," it seems desirable in recording new traits to indicate in so far as possible the circumstances under which they arose. It has to be remembered, however, that apart from statistical probabilities it is still impossible to distinguish between mutations that have been induced and those that may have occurred spontaneously. In the present instance a deliberate attempt had been made to induce mutations and the *di* trait is among those that appeared in course of the experiment.

Duplicate incisors were first noticed in two males descended from mice which in earlier generations had been treated with nitrogen mustard (HN_2). The first mouse so treated, a *ruru* female from a general purpose stock, was given a barely sublethal injection shortly after mating and while the copulation plug was still present. She gave birth to three young whose descendants were interbred in various combinations to produce numerous lines of descent, some of which had heritable anomalies. All the members of one apparently normal family among these descendants were given injections of nitrogen mustard that would ordinarily have been lethal but which in this instance were accompanied by protective doses of cysteine. Most of these mice survived and their descendants were

FIGURE 1.—Accessory incisors in a 20-day old female mouse. It may be noted that the extra teeth extend upward and outward from behind the normal ones. At 32 days the accessories had been broken off and were not apparent on superficial inspection, but by 67 days they were again prominent. When the mouse was killed and autopsied at 120 days the extra teeth were both present, but small and pointed at their tips.

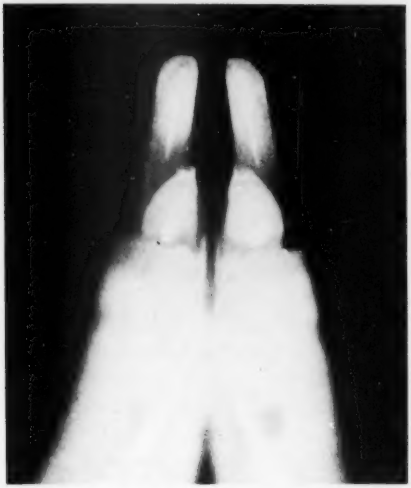

FIGURE 2.—Lingual aspect of the tip of a lower jaw at about the time when the extra teeth erupt through the gums.

designated as the "J line." Crossed to long inbred C57Bl/6 mice this J stock produced vigorous hybrids. One F_2 female (No. 100) from such a cross was mated to her father (F_1) and observed to have a copulation plug on March 4, 1954. On March 15 she was injected intra-abdominally with 0.05 cc of a solution made by dissolving 10 mg of HN_2 in 20 cc of 0.75 percent NaCl in distilled water. The mouse showed no observable effects of the injection and on March 24 gave birth to nine young, four females and five males, all apparently normal. However, since we have found that similar treatment of females in comparable stages of pregnancy ordinarily results in abnormalities among the young (DANFORTH and CENTER 1954), it may be assumed that in this case also the fetuses were, in effect, treated *in utero*. Two males were discarded and the rest of the litter kept for further observation.

On June 8, 1954, one pair of these 'treated-in-utero' mice produced three young, two females and a male, which were retained as a breeding group. They in turn gave rise to a considerable number of offspring, but no significant anomalies were noticed until January 12, 1955, when among 13 adolescent young DR. ELIZABETH CENTER, to whom the writer is much indebted for cooperation in this study, found two males each with an extra lower incisor on one or both sides. This first observed appearance of the trait was thus in the second generation from young that had been treated *in utero*. The two males mated to three of their full or half sibs are ancestral to all *di* mice that have since appeared. It may be noted here that among the antecedents of these mice no individual had ever been treated more than once during its lifetime.

Laterality in expression of the trait

Since the *di* trait shows some variation in the form of its manifestation, most of the young have been recorded on the basis of their phenotypic appearance, *B* indicating those in which an extra tooth was clearly present on both sides, *R* those in which it could be detected on the right side only, *L* on the left side only, and *O* on neither side. All young classified as *B* unquestionably were such, but an occasional one in which a tooth had been pushed out of its socket or broken off beneath the gums may have been assigned to the wrong group. In an effort to avoid errors that might arise in this way, almost all animals not to be used for breeding were sacrificed before reaching six weeks of age and their jaws cleaned in a sodium hypochlorite solution (commercial "PUREX"), making it possible to confirm or correct the original diagnoses. This rather laborious procedure, involving the preparation of many hundred specimens, corrected a few oversights but served chiefly to check the accuracy of direct observations on living young. It should be mentioned, however, that a few extra teeth were mere spicules that could not have been detected in living animals and that embryological evidence indicates that an occasional accessory enamel organ fails to produce any recognizable tooth at all.

There is some indication that an extra tooth is more likely to be present on the right side than on the left. Among 129 jaws showing asymmetry, the extra tooth was larger on the right side, or present on that side only, in 74 cases, while the opposite was true in only 55 cases.

Breeding tests

Distribution of the trait and grades of its expression in progenies of inbred, outcrossed and extracted parents are shown in the table, where there are probably very few errors in classification of the young, but where an occasional parent listed as *R*, *L*, or *O* may have actually belonged to a grade higher than the one indicated. It may be mentioned at this point that after the breeding tests were well under way, and most of the data presented here had been accumulated, the colony fell victim to an epidemic which decimated some lines and curtailed the reproductive performance of others. This has left some obvious hiatuses which will need to be filled in when the surviving stock can be brought back to normal. In the meantime it seems desirable to present available data on the approximately 2000 offspring whose records are unimpaired.

A. *Outcross matings.* One of the original *di* males, and later inbred individuals of both sexes, served for outcrossing to four different lines which may be briefly characterized.

A-1. The "Swiss" referred to in the table were supplied by another department which had obtained albino stock from several sources, one of which specializes in the Webster strain. It is probable that more or less remote ancestors of some of these mice had been subjected to nitrogen mustard or other presumed carcinogenic agents.

TABLE 1

Distribution of the di *trait among the offspring from various types of mating*

A. Outcrosses

Matings	Offspring *B*	*R*	*L*	*O*	Totals
1. *di* × Swiss					
F_1	0	0	0	27	27
F_2	0	0	0	276	276
BC	10	10	11	282	313
2. *di* × C57Bl					
F_1	0	0	0	97	97
F_2	2	3	3	286	294
BC	11	6	7	100	124
3. *di* × P-Jax					
F_1	0	0	0	7	7
F_2	0	0	0	34	34
BC	2	2	0	53	57
4. *di* × "100"					
F_1	2	0	1	167	170
F_2	0	3	0	48	51
5. Totals: 1-4					
F_1	2	0	1	298	301
F_2	2	6	3	644	655
BC	23	18	18	435	494
Totals	27	24	22	1377	1450

B. Inbred matings

Matings	Offspring *B*	*D*	*R*	*L*	*O*	Totals
1. *B* × *B*	204	..	12	10	17	243
2. *D* × *D*	25	1	6	4	2	38
3. *B* × *D*, *R* or *L*	26	..	7	2	12	47
4. *R* × *R*, *R* × *L*, *L* × *L*	17	..	6	5	12	40
5. *B* × *O*	8	10	1	1	21	41
6. *D* × *O*	8	6	1	0	18	33
7. *O* × *O*	11	0	3	1	8	23
Totals	299	17	36	23	90	465

8. Percentages of *B* young from matings 1-4 above	
a. Both parents bilateral	83.9±1.6
b. Both parents *D*	65.8±5.2
c. One parent *B*, one unilateral	53.3±4.9
d. Both parents unilateral	42.5±5.3

C. Extracted group

Matings	Offspring *B*	*D*	*R*	*L*	*O*	Totals
1. *R* × *R*	11	..	4	2	34	51
2. Filial *B* × *B*	16	..	1	1	3	21
3. Filial *B* × *O*	2	..	1	0	16	19
4. Filial *R* × *O*	0	..	1	2	6	9
5. Filial *B* × (F_2)*O*	4	..	0	1	2	7
Totals	33	..	7	6	61	107

B, duplicate incisors on both sides; *R*, a duplicate on the right side only; *L*, duplicate on the left side only; *O*, no duplicate on either side; *D*, at least one duplicate, but record as to side incomplete. C-1 shows offspring of *R* mice derived from *di* × Swiss, backcrossed to *di*; C-2 and ff. give results from mating some of these young.

A-2. C57Bl/6 mice were obtained from the Roscoe B. Jackson Memorial Laboratory and were from at least the 42nd generation of brother-sister matings, or from one or two later generations in which matings may have been between parent and offspring. One subdivision of the group, however, was derived from the mating of a *di* female to a C57 male whose grandmother had received an injection of nitrogen mustard during pregnancy. This subgroup accounts for 79 of the 294 young in F_2. One of these was *di*, the other 78 were normal.

A-3. P-Jax, a strain homozygous for a number of recessive genes, was also obtained from the Jackson laboratory, where over 50 generations of brother-sister matings had been recorded.

A-4. Mice designated as "100" were all descended exclusively from the surviving seven young produced by female No. 100 following her treatment with nitrogen mustard. None of these came directly from the *di* subline, but one of the progenitors of that line may have been ancestral to some of those in this group.

Section A of the table shows the observed frequencies of *B*, *R*, *L*, and *O* individuals among the young that could be examined in the F_1, F_2 and BC_1 generations from crosses A-1, A-2 and A-3, and the F_1 and F_2 generations form the A-4 cross. Data from F_3, BC_2, and other less critical matings are not included, but they appear to be in essential agreement with the figures presented. Summarizing these figures (A-5), and counting all *B*, *R* and *L* mice as *di*, it appears that in F_1 there was a total of 301 young, of which three (all from A-4) were *di*, giving an over-all incidence of about one percent. In F_2 there were 655 young of which 11, or approximately 1.7 percent were *di*, and in BC_1 494 young with 59, or about 12 percent *di*.

B. *The inbred line.* All the mice in this series were descended exclusively from one or both of the original *di* males mated to one or more of their three sibs. Section B of the table shows the types of matings that were made and characteristics of the young that were produced. In this section, *D* indicates that the animals in question were definitely *di* but with the exact grade unrecorded. Any *D* mouse may have been *B*, *R* or *L*. A summary of the figures shows that of 368 young from *di* × *di* matings, 325, or approximately 88 percent were *di*, while of 74 young from parents one of which was *di* the other *O*, but of *di* ancestry, 47 percent were *di*. There were also 23 young from parents both of which had been classified as *O*, and among them 16 were *di*. A few individual matings selected from these series sufficed to show that any type of parental combination may at times produce *B*, *R*, *L* or *O* offspring.

C. *Progenies from matings of extracted* di *parents.* A few extracted *di* mice were interbred and distribution of the trait among their young roughly paralleled that in the inbred line. The group selected for the table came from three *R* young born at the same time but to different parents. It may be noted that among their descendants all grades of the trait appeared.

Interpretation of the findings

A. *Data from outcross matings.* The figures are sufficient to show that the *di* trait is hereditary, and that it is not an ordinary dominant. Its first observed appearance, however, coincided exactly with what might have been anticipated if a single recessive mutation had occurred in the germinal tissues of one of the embryos treated *in utero*. If such a mutation did in fact occur, the low frequencies in the F_2 and BC generations could conceivably be due either to reduced viability of homozygous recessives or to some inequality in the number or functional potency of germ cells carrying alternative alleles. That such alleles are not always distributed impartially to developing germ cells might be inferred from studies on "affinity" (MICHIE 1953, 1955; WALLACE 1953) and the observations of DUNN (1957) and several of his associates who have found what appears to be an unaccountably high incidence of sperm cells carrying specific lethals in a number of strains of wild mice. The alternative to some such hypothesis is that the *di* trait must be attributed to the effects of more than one independent gene.

A *BC/F_2 index* is useful in testing such postulates. The index, representing the

ratio between the incidence of a trait in BC and in F_2, derives its value from the fact that, with reference to each particular gene, an F_1 individual can produce two kinds of germ cells while a homozygous recessive can produce only one. This circumstance gives rise to the expectation that, in the absence of complicating factors, the incidence of a recessive trait in BC will always be the square root of its incidence in F_2, irrespective of the number of contributing genes that may be involved. By means of this index it is possible to utilize simultaneously the results from two critical types of mating in testing agreement between observed and expected figures.

In the present instance, if the trait is a semilethal in the sense that a disproportionate number of recessives are lost between zygote formation and the time when they can be counted, the fact should be reflected in the BC/F_2 index. Since the 'normal' incidence of a recessive is $1/(3+1)$ in F_2 and $1/(1+1)$ in BC, the BC/F_2 index is ordinarily $(3+1)/(1+1)$, but if a relatively smaller proportion, n, of the recessives survive, the index becomes converted to $(3+n)/(1+n)$, a fraction whose value must always lie between 2, when $n=1$ (all recessives surviving) and 3 when $n=0$ (no recessives surviving). In other words, whatever the extent of postzygotic lethality due to any one gene the BC/F_2 index must always fall between 2 and 3 unless entirely extraneous factors are involved. The *di* figures yield an index of approximately 7, (i.e., 0.12/0.017) which makes it extremely unlikely that any one gene can account for the shortage of *di* individuals.

Even though an explanation of the numerical deficiencies must apparently be sought in pre- rather than postzygotic stages, the BC/F_2 relation may still be of significance. If germ cells with alternative alleles are produced, or function, in unequal numbers, p may be allowed to stand for the relative number or potency of those carrying the dominant allele and q for those with its recessive counterpart. Using this notation, the expected incidence of a simple recessive in F_2 is $[q/(p+q)]^2$, and in BC $q/(p+q)$. On the basis of the total figures for F_2 (A-5), where 0.017 may be presumed to correspond to $[q/(p+q)]^2$, the value of q in terms of p is about 0.129. For BC it is about 0.119, and the weighted average is 0.125+. This value of q leads to an expectation of 10 *di* individuals instead of the observed 11 in F_2 and 61 instead of 59 in BC, an agreement as close as could be expected if the hypothesis were known to be correct.

Finally, if instead of a single mutant gene with reduced effectiveness, the trait is presumed to be due to multiple recessives of the usual sort, the BC/F_2 index should be 2^n, and the calculated index of 7+ is so close to 2^3 as to suggest that three pairs of alleles might be involved. This would lead to essentially the same expectations as the prior assumption, and it must be concluded that in this case the data from outcross matings are not competent to differentiate between expectations based on a single recessive gene with $\frac{1}{8}$ the potency of its normal allele and those based on the necessary presence of three contributing but independent normal recessives. Except for the circumstances surrounding the first appearance of the trait, there would have been little hesitancy in accepting the second alternative.

B. *Data from the inbred line.* Figures from the inbred line indicate that when both parents have *di* ancestry *di* young are to be expected, and that the grades of expression in the young are related to those in the parents. As indicated above, combining the data from matings B-1 to B-4, where both parents in each case were *di*, gives a total of 368 young of which 325 (88 ± 1 percent) were *di*, while matings B-5 and B-6 in which one parent was *di*, the other *O* but of *di* ancestry, show 74 young of which 35 (47 ± 4 percent) were *di*. In the small B-7 group, some $O \times O$ matings are recorded as having produced *di* young, a seeming inconsistency that may be due to erroneous classification of one of the parents.

That a bilateral expression of the trait mirrors some kind of cumulative genic effect is suggested by the relative frequencies indicated under B-8 in the table, where a progressive decrease in incidence is shown from 84 percent when both parents were *B* to 43 percent when each parent showed the trait on one side only. On the whole, the various data from inbred matings supplement the evidence that multiple genes are involved in production of the *di* trait.

C. *Data from matings of extracted individuals.* Among the young from the extracted group, occurrence of the *di* trait roughly paralleled its occurrence in the inbred line, which seems to indicate that the essential genes are capable of independent segregation, or at least are not closely linked.

DISCUSSION

In view of the conditions under which the *di* trait was first observed, the possibility that it could be accounted for by a single gene mutation has called for special consideration. The BC/F_2 index effectually rules out any likelihood that a single mutant gene could be semilethal to an extent that would account for the observed figures, and as between a single recessive with reduced efficiency and multiple recessives with normal potencies, the evidence on the whole favors the latter, with indications that the number of critical genes is probably three. Nevertheless there is an appreciable margin of deviations from expectations calculated on either of the assumptions. Such deviations, commonly attributed to incomplete penetrance and expressivity (cf. LANDAUER 1955), vary with the type of mating. The $B \times B$ matings indicate a penetrance of 93 percent, and it is clear that at least part of the missing seven percent can be accounted for by failure of some of the accessory enamel organs to produce teeth. Postulating a fourth effective gene would throw the expected and observed figures far out of line. In view of these considerations, the best inference that can be drawn from the available data would seem to be that the *di* trait is dependent on three separate complementary genes.

With the preponderance of evidence indicating a three-gene combination, the problem arises as to how such a complex could have come about. Even though our knowledge of the action of chemical mutagens is meager (AUERBACH 1949), it would seem unlikely that a single treatment could cause simultaneous changes in three, even functionally related, genes. A more plausible inference is that two of the necessary genes were already in the germplasm when the final *di* mutation

occurred. Such genes might have come from either the C57 or the J ancestry, and the figures from A-2 and A-4 afford some, but very slight, support for such a view. Whether the final *di* mutation was spontaneous or induced can not be answered with certainty, although the manner of its first appearance makes induction seem probable, as do some of the mutations observed by FALCONER, SLIZYNSKI and AUERBACH (1952) following treatment with nitrogen mustard. That the mutation happened to occur in a milieu which permitted it to be expressed is possibly no more than a coincidence. That this milieu, however, is not merely a kind of generalized background but a matter of only two, or at most three, specific genes seems to be clearly indicated.

Whatever other functions they may have, the alleles of these background genes tend to submerge the effects of the new mutation and in that sense contribute to a kind of "genetic homeostasis" (LERNER 1954). In such a case as the present one, phenotypic stability could depend on concurrent action of both dominant and recessive genes, and figures in section B of the table suggest that, in the presence of the other two, at least one of the contributing genes may act as a semidominant. As yet no independent pleiotropic effect of any one of these three genes has been detected, and this makes it impossible at present to differentiate between them. The possibility that some undetermined form of chromosomal aberration may account for deficiencies in appearance of the trait is not supported by evidence thus far available.

Behavior of the *di* trait provides an interesting sidelight on the possible fallibility of conventional tests designed to detect mutagenic effects. FALCONER (1949) has presented a valuable discussion of the number of suspected gametes that can be adequately tested and the reliability of tests when a possible recessive is involved, but in cases such as presented by the *di* complex the results of such tests might easily be misleading. When one of our *di* males was outcrossed to unrelated stocks, 189 F_1, F_2 and BC_1 young were raised to the proper age and examined before the first *di* individual appeared among them. If female sibs of this original male had not been available for further tests it is probable that the trait would have been regarded as sporadic with no hereditary background. It is not likely that behavior of the *di* trait is unique and, if it is not, it becomes probable that in mice and other mammals many traits that are ordinarily regarded as sporadic do in fact have a definite and analyzable, even though complex, genetic background.

SUMMARY

A new trait, *di* (duplicate incisors), appeared in the second generation from mice treated *in utero* by injecting the pregnant mother with nitrogen mustard. When *di* mice were outcrossed, frequency of the trait was found to be low in the F_2 and BC generations; 1.7 and 12 percent respectively. A BC/F_2 index shows that the low incidence can not be accounted for on the assumption of a single semilethal mutant gene, but that it could be explained by postulating a gene which reduces the zygote-forming capacity of any germ cell which happens to carry it. The same figures could be explained equally well by assuming that

instead of one defective gene there are three normal recessives all of which must be present to insure appearance of the trait. Results from the inbred matings favor the second alternative and provide some evidence that one or more of the three genes may act as a semidominant when the other two are homozygous. A small part of the numerical deficiency is due to an unexplained failure of certain accessory enamel organs to produce teeth.

The sudden appearance of a trait apparently dependent on three different genes presents problems which at present can not be answered. It is conceivable, but extremely unlikely, that three mutations occurred simultaneously in the same embryo. It is hardly more likely that complementary mutations occurred in the two embryos which were to become ancestral to the *di* line. Instead, it seems probable that two of the mutations had already occurred, either spontaneously or as the result of treatment in earlier generations, and remained latent until the third one brought all of them to light. With three separate genes, none of which can as yet be individually identified, the group as a whole will have to be referred to simply as the *di* complex until such time as one of the genes becomes revealed through some distinctive effect of its own.

Experience with the *di* complex serves to emphasize the possible inadequacy of tests for mutagenic effects when such tests are predicated on the assumption that only simple monofactorial traits need be looked for. In an animal with the genetic complexity of the mouse it is probable that there are many latent genes whose presence would never be detected in the absence of an additional potentiating mutation. Whenever the number of genes necessary for the expression of a trait reaches three or more the genetic situation becomes difficult to analyze. Individually ineffective hidden genes may presumably accumulate in appreciable numbers and, in infrequent combinations, be responsible for a considerable part of the unexplained manifestations which are usually regarded as sporadic.

LITERATURE CITED

AUERBACH, C., 1949 Chemical mutagenesis. Biol. Rev. **24**: 355–391.

CENTER, E. M., 1955 Postaxial polydactyly in the mouse. J. Heredity **46**: 144–148.

DANFORTH, C. H., and E. M. CENTER, 1954 Nitrogen mustard as a teratogenic agent in the mouse. Proc. Soc. Exp. Biol. Med. **86**: 705–707.

DUNN, L. C., 1957 Evidence of evolutionary forces leading to the spread of lethal genes in wild populations of house mice. Proc. Natl. Acad. Sci. U.S. **43**: 158–163.

FALCONER, D. S., 1949 The estimation of mutation rates from incompletely tested gametes and the detection of mutations in mammals. J. Genet. **49**: 226–234.

FALCONER, D. S., B. M. SLIZYNSKI, and C. AUERBACH, 1952 Genetical effects of nitrogen mustard on the house mouse. J. Genet. **51**: 81–88.

LANDAUER, W., 1955 Recessive and sporadic rumplessness of fowl: effects of penetrance and expressivity. Am. Naturalist **89**: 35–68.

LERNER, I. M., 1954 *Genetic Homeostasis*. vii + 134 pp. John Wiley and Sons. New York.

MICHIE, D., 1953 Affinity: a new genetic phenomenon in the house mouse. Evidence from distant crosses. Nature **171**: 26–27.

1955 "Affinity." Proc. Roy. Soc. London B **144**: 241–259.

WALLACE, M. E., 1953 Affinity: a new genetic phenomenon in the house mouse. Evidence from within laboratory stocks. Nature **171**: 27–28.

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